Paired Pulse Electrical Stimulation In Human Intractable Focal Epilepsy

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ABSTRACT

OBJECTIVE: The purpose of this study was the identification of synaptic changes related to epileptogenesis in patients investigated with intracranial recordings during presurgical assessment.

HYPOTHESIS: The following hypotheses were tested:

1. Suppression, depression or facilitation is related to seizure onset area.
2. The removal of the cortex showing suppression, depression or facilitation is associated with better surgery outcome.

METHODS: A total of 79 patients with intractable focal epilepsy in whom intracranial electrodes were implanted for assessment prior to epilepsy surgery were analysed, using paired pulse electrical stimulation. The amplitude of the response elicited from the first pulse (1^{st} response) was compared with the amplitude of the response elicited from the second pulse (2^{nd} response). Depending on if the 2^{nd} response was absent, of reduced, increased or similar amplitude to that of the 1^{st} response four different conditions were emerged: a) suppression, b) depression, c) facilitation, or d) no change.

RESULTS: The following results were noted: a) Suppression showed better relation with SO lobe than depression and facilitation b) In patients with focal onset, suppression was observed in the area surrounding the focus and c) Resection of the suppressed areas was found to be an unreliable marker of surgical outcome.

DISCUSSION: The distribution of suppression in seizure onset lobe and more specifically in the area surrounding the focus can be of particular interest to identify the epileptogenic lobe and to study the pathophysiology of human focal epilepsy.

This PhD is dedicated to my family.

Their support all these years was invaluable.

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A. Epidemiology of epilepsy

Incidence: Epilepsy is the 2nd most common chronic neurological disorder (WHO 2006) with an incidence between 32 and 71 (mean 46) per 100.000 people per year (Hirtz et al 2007). In Europe and USA, the incidence is 50-55 cases per 100.000 people per year (Forsgren et al 2005). In developing countries, incidence may be as high as 100-190 cases per 100.000 per year (Bell and Sander 2001). For focal epilepsies the annual incidence is approximately 35 cases per 100.000 (Zarrelli et al 1999)

Prevalence: Prevalence has been estimated at 4-10 cases per 1000 people, which translates into more than 50 million patients worldwide suffering from this disorder (Kwan and Brodie 2000, Unnwongse et al 2010, Bell and Sander 2001). At least 3.1 million Europeans (6 per 1000 per year) have active epilepsy (Forsgren et al 2005). Approximately, 25% of patients are children, 55% are adults and 15-20% are elderly people (Forsgren et al 2005). The prevalence of persistent seizures in symptomatic epilepsy amounts to approximately 40 percent (Kwan and Brodie 2000).

B. Human focal epilepsies

The most common human focal epilepsies are temporal or frontal in origin. According to the 1989 ILAE Classification of Epilepsy and Seizures (Commission on Classification and Terminology of the International League Against Epilepsy 1989), temporal and frontal lobe epilepsies can manifest as simple partial seizures, complex partial seizures, secondarily generalized seizures or a combination of these seizure types.

B.1. Temporal lobe epilepsy

Temporal lobe epilepsy is the most common focal epilepsy in humans.

In simple partial seizures, there is no impairment of consciousness. Simple partial seizures in temporal lobe epilepsy may manifest as feeling arising epigastric sensations, fear, and déjà-vu among the most common symptoms, with auditory illusions, olfactory and gustatory hallucinations less frequently reported.

In complex partial seizures, consciousness is impaired. Simple partial seizures may precede complex partial seizures. Motor arrest with staring, simple (e.g. oroalimentary) or complex (e.g. inappropriate behaviour) automatisms and autonomic phenomena like cardiovascular symptoms (tachycardia, bradycardia, and asystole), respiratory arrest, mydriasis, nausea and vomiting can be seen. Postictal confusion with amnesia follows the complex partial and secondarily generalised tonic-clonic seizures. Certain symptoms, such as postictal aphasia are important for lateralization. Ictal speech suggests non-dominant hemisphere seizure onset while unilateral motor dystonia suggest seizure onset contralataral to the dystonia.

Seizures in temporal lobe epilepsy, most often arise from the mesial structures (amygdala, hippocampus, parahippocampal gyrus) and the most common cause is temporal sclerosis. Less common causes include tumours (i.e. DNET), malformations of cortical development and vascular abnormalities.

Scalp EEG recordings obtained in the interictal period often show temporal slowing and unilateral or bilateral independent epileptiform discharges. Ictal scalp EEG consists of diffuse slowing or focal EEG rhythms (4-7 Hz) usually starting over anterior temporal regions (Alarcon et al, 2001). High resolution MRI is mandatory to identify mesial temporal sclerosis or other underlying causes.

Approximately 30% of patients with temporal lobe epilepsy do not respond to drugs and surgery is a treatment alternative (Zimmerman and Sirven 2003). Anterior temporal lobectomy is the most frequent surgical approach which includes removal of the hippocampus and parahippocampal gyrus in addition to various degrees of neocortical removal depending on hemisphere dominance (Selway, 2012; Zimmerman and Sirven 2003). Anterior temporal lobectomy is associated with a 70% likelihood of seizure control. Amygdalohippocampectomy is an alternative, which is thought to affect memory to a less degree. Good postsurgical seizure control seems to be related to the extent of resection of the hippocampus and parahippocampal gyrus. Lesionectomy can be

performed according to the location of the lesion. A tailored cortical resection as defined with the aid of intracranial recordings can be performed when the focus is less precise (patients with normal MRI or neocortical seizure onset).

B.2. Frontal lobe epilepsy

Frontal lobe epilepsy is characterised by short seizures with minimal postictal confusion, prominent motor phenomena and complex, bizarre or frantic automatisms. The frontal lobe is the largest lobe, containing nearly half of the cerebral cortex, and is subdivided into several anatomical areas, which give rise to their own seizure semiology. Depending on the site of origin, seizure characteristics may include asymmetric or fencing posturing (supplementary motor cortex), autonomic symptoms (cingulate cortex), forced thinking and adverse head and eyes version (frontopolar region). Olfactory hallucinations and illusions are seen in seizures arising from the orbitofrontal cortex, while mastication, salivation, speech arrest, epigastric aura and fear are symptoms arising from the opercular area.

Malformations of cortical development, tumours and vascular abnormalities are among the commonest causes. MRI is useful in identification of such structural abnormalities.

The interictal scalp EEG may be normal or show frontal slowing, frontal epileptiform discharges or bilateral/generalised discharges. The ictal scalp EEG can show unilateral, or often bilateral, fast activity mixed with spikes and sharp waves.

However, precise localization of the epileptogenic area in frontal lobe epilepsy is often a challenge for the following reasons: a) Numerous connections with temporal and parietal lobes as well as the contralateral frontal lobe result in the fast seizure propagation (Binnie et al 2003); and b) Widespread distribution of the epileptogenic area. The consequences of these characteristics are that the clinical manifestations may suggest temporal or parietal lobe epilepsy; and if symptoms do suggest frontal lobe epilepsy, laterality might be unclear.

As opposed to temporal lobe epilepsy, no standard surgical procedures are available (Alarcon et al 2009).

Frontal cortical resection and lesionectomy are the most common techniques used, and often tailored to each patient. In cases where the epileptogenic cortex includes eloquent areas, multiple subpial transection (MST) can be performed (Morrell 1989).

C. Refractory Epilepsy and epilepsy surgery

More than a third of epilepsy patients worldwide eventually develop refractory epilepsy, i.e, patients where seizures are poorly controlled by medication (Kwan-Brodie 2002, Mohanraj-Brodie 2006). Between 5% and 50% of patients with refractory epilepsy can benefit from surgical treatment (Ryvlin and Rheims 2008). Such a wide percentage range is probably due to differences in the selection criteria of candidates during pre-surgical evaluation, to the gap between the number of potential candidates and the number of operated patients (Engel et al 2003), and to the long delay (of up to 20-25 years) between referral for surgery and operation (Yoon et al 2003, Janszky J. et al 2005). Taking into consideration that a successful surgical outcome depends primarily on the epilepsy syndrome (Engel 1996), it is imperative that presurgical evaluation should be based upon common standards and on the recognition that late referrals should be avoided, as they are associated with poorer results. These two factors remain of paramount importance as they would provide reliable presurgical predictors of outcome, which could result in improving post-surgical seizure control.

The most effective surgical method for the treatment of focal epilepsy is the surgical resection of the areas involved in originating the seizures. The surgical procedure of choice depends on seizure type, location and extension of the underlying pathology, and its relation to functionally relevant cortex. A variety of methods are available for the identification of seizure focus, including interictal and ictal scalp electroencephalography, magnetic resonance, positron emission tomography and neuropsychology. However, approximately 25-30% of patients operated in the best centres do not improve substantially after surgery (Alarcon et al, 2006; de Tisi et al, 2011). The reasons for surgical failure are unclear. Presurgical identification of patients who will suffer poor outcome is difficult even with the use of modern neuroimaging and

intracranial electroencephalography. In practice, the surgical decision depends on presurgical estimations of the likelihood of patients' improving with surgery.

A number of pre-operative predictive factors for seizure control after epilepsy surgery have been described. In the general population of patients operated for epilepsy, positive prognostic factors (those associated with better post-surgical seizure control) include the extent of surgical resection, presence of lesions on MRI, mesial temporal sclerosis, tumour pathology, EEG/MRI concordance, and febrile convulsions (Beghi and Tonini, 2006; Tonini et al, 2004), removal of regions showing abnormal responses to electrical stimulation in intracranial recordings (Valentin et al., 2002, 2005a,b and Flanagan 2009) and the nature of the surgical procedure, with temporal lobectomy and hemispherectomy resulting in better outcome (Zupanc et al., 2010). In the long term, the use of selective procedures rather than lobar resections appears to be a positive predictive factor (Dunlea et al., 2010). Negative predictive factors (those associated with poor post-surgical seizure control) include the need for intracranial monitoring (Beghi and Tonini, 2006; Tonini et al, 2004) and, for long-term seizure control, performing a temporal neocorticectomy, (Dunlea et al., 2010). Interestingly, normal neuroimaging does not seem to be a negative prognostic sign (Alarcon et al, 2006).

As expected, most studies discussed above contain a high proportion of patients with temporal lobe epilepsy, which is the most common focal epilepsy amenable to surgical treatment. A number of preoperative positive prognostic signs have been described specifically for extra-temporal epilepsy, including short duration of epilepsy (Elsharkawy et al., 2008a,b,c), age at surgery below 30 (Elsharkawy et al., 2008a,b; only marginally by Ansari et al., 2010), tumoral pathology (Elsharkawy et al., 2008a), localised lesion on MRI (Elsharkawy et al., 2008a,b; Ferrier et al, 1999; Lee et al, 2008; Yun et al., 2006), localised seizure onset on EEG (Yun et al., 2006), interictal discharges ipsilateral to surgery (Elsharkawy et al., 2008b), abolition of seizure-like interictal discharges in focal cortical dysplasia (Ferrier et al, 2001), use of invasive monitoring (Elsharkawy et al., 2008c), presence of complex partial seizures (Ansari et al., 2010) and concordance of multiple tests (Jeha et al., 2007; Kun et al., 2005; Lee et al, 2008, 2005; Yun et al., 2006). Negative predictors in extratemporal epilepsy include previous surgery, convulsive seizures and auditory aura (Elsharkawy et al., 2008c), and the use of subdural mats in frontal lobe epilepsy (Elsharkawy et al., 2008b). Interestingly, removal of all histological

abnormalities or areas showing sporadic interictal discharges does not affect seizure outcome in frontal lobe epilepsy (Ferrier et al, 2001).

The absence of improvement in seizure control after epilepsy surgery could be explained by the limitations in our methods to identify epileptogenic cortex pre-surgically. In the present thesis, I explore the value of several aspects of EEG responses to electrical stimulation of the human cortex via intracranial electrodes as markers for the identification of epileptogenic cortex, and discuss their value during presurgical assessment of epilepsy.

D. Presurgical evaluation with intracranial recordings.

D.1. Identification of epileptogenic zone (EZ)

The principal aim during presurgical evaluation is the identification of the epileptogenic zone which is defined as *"the zone that generates seizures and a complete resection of it would render the patient seizure free"* (Unnwongse et al 2010). Despite modern technical advances in surgical procedures and pre-surgical assessment over recent decades, the overall success rate of resective surgery for epilepsy remains at about 70% (Alarcon et al 2009). The reasons for this are unclear, but may be related to present shortcomings in the identification and characterisation of the epileptogenic zone. In addition to the epileptogenic zone, the following related areas are also involved in the pathophysiology of focal seizures in humans (Unnwongse et al 2010, Gelziniene et al 2008, and Ryvlin and Rheims 2008):

a) The *symptomatogenic zone:* the area responsible for the symptoms.

b) The *functional deficit zone*: the zone that is functionally abnormal during interictal period

c) The *epileptogenic lesion*: the structurally abnormal area

d) The *irritative zone*: the zone generating interictal epileptiform discharges

e) The *seizure onset* area: where seizures originate

The definition of the epileptogenic zone requires performance of surgery and correlation with surgical outcome. For this reason, we will prefer the term *epileptogenic cortex* to designate cortex involved in originating seizures in situations when surgery is not considered or has not been performed.

D.2. Conventional surgical evaluation of epileptic patients

The definition of the epileptogenic zone implies that it can only be identified post-surgically (it is contained within the resected specimen only if the patient becomes seizure free). However, there are non-invasive and invasive pre-surgical tests that are helpful in the identification of epileptogenic zone (Ryvlin and Rheims 2008 and Gelziniene et al 2008).

- *Non-invasive methods:* This is the 1st phase of assessment and include a detailed history, neurological examination, neuropsychological testing (sometimes including the amytal test), scalp video-EEG telemetry and structural (MRI) and functional imaging (PET, SPECT, fMRI). The location of eloquent context may need to be investigated if the epileptogenic cortex is to cortical areas involved in speech, motor, sensory or memory function.
- *Invasive methods:* Ictal EEG recordings with intracranial electrodes may be required when non-invasive tests are inconclusive (European Federation of Neurological Societies Task Force. 2000).

D.3. Intracranial EEG strategies

When non-invasive methods of presurgical assessment do not identify a single source for the patients, recording of seizures with intracranial recordings remains one of the most reliable methods to identify epileptogenic cortex. The gold standard for this is the identification focal EEG changes at seizure onset located in the same region during at

least 4-5 seizures. Withdrawal or reduction of antiepileptic medication during may be necessary to achieve this goal.

As cortical sampling with intracranial electrodes is necessarily limited, the information provided by intracranial recordings can be ambiguous if electrodes are not implanted near epileptogenic cortex. Seizures can originate in an area not covered by electrodes and then secondarily propagate to areas close to the implanted electrodes, potentially giving the wrong impression that seizure originate in areas close to the implanted electrodes.

At present, about 50% of patients assessed for epilepsy surgery at King's College Hospital require chronic implantation of intracranial recordings with video-telemetry to identify the cortical areas originating seizures.

Since epileptic seizures are thought to arise from an increase in cortical excitability or decrease in inhibition, identification of the epileptogenic zone can be achieved by the measurement of cortical excitability using with EEG responses to cortical stimulation via intracranial electrodes. Single pulse electrical stimulation (SPES) has been used with this aim in our center for over 12 years. During SPES, brief single pulses (1 ms) are applied in the cortex via a pair of contiguous intracranial electrodes and EEG responses are recorded by the remaining electrodes. The location of the elicited responses has been compared to seizure onset area (Valentin et al 2002, 2005a, 2005b, Flanagan et al 2009).

Valentin et al (2002) studied 45 patients with intractable epilepsy admitted at King's College Hospital for assessment prior to epilepsy surgery. Two types of responses were elicited by single pulse electrical stimulation: a) early and b) late responses. Responses were compared to SO area. No specific relation was identified between early responses and SO areas, as they were seen in most areas. However, a strong relation was found between SO areas and late responses. The authors concluded that late responses could identify hyperexcitable epileptogenic cortex.

In an additional study Valentin et al (2005) examined the association between delayed responses and seizure outcome in 40 patients who had epilepsy surgery. Twenty-two patients had late responses in resected areas (96% with good outcome), 7 patients had late responses in resected and non-resected areas (71% with good outcome), 3 had late

responses in non-resected areas (none with good outcome) and 8 patients had no late responses to SPES (62.5% with good outcome). Similar findings were observed by Flanagan et al (2009) in 35 children. Delayed responses were noted in 54% of cases.
A favorable outcome (Engel grades 1 or 2, see Methods section E.3.2. Epilepsy Surgery-Surgical outcome) was observed when the entire area with late responses was removed.

The above studies suggest that SPES is useful in the identification of epileptogenic cortex with enough specificity to become a clinical tool. At King's College Hospital, SPES is an integral part of the intracranial EEG strategy during presurgical evaluation of patients for epilepsy surgery.

D.4 Functional testing

As mentioned above, identification of the epileptogenic zone is of paramount importance for epilepsy surgery. Occasionally, the epileptogenic zone is close to the eloquent cortex. Identification of the areas with these vital functions is then a prerequisite.

Functional mapping (stimulation) using mat electrodes, is a standard method to identify these areas. Stimulation is performed through neighboring pair of electrodes with progressively increasing steps in current intensity until: a) clinical signs of the frontal, parietal and temporal lobe are noted like muscle twitching, tingling sensation or speech arrest respectively b) afterdischarges are seen on the EEG and c) a maximum intensity level of 10 mA is reached (Alarcon and Binnie 1995). Signs with afterdischarges on the EEG are of limited value as these signs can be due to propagation. On the other hand, if these signs are not accompanying by afterdischarges, they are of localizing value as they would indicate that stimulation is being carried out close to eloquent cortex. For this reason the concordance between spontaneous and electrically induced seizures was explored quite early (Wieser et al 1979, Bernier et al. 1990, Chauvel et al. 1993, Schulz et al. 1997, Alarcon 2009). Wieser et al found that in 77% of temporal lobe patients there was a concordance between spontaneous and electrically induced seizures (Wieser et al 1979). Chauvel (1993) also found a high correlation between onset zone for spontaneous seizures and sites stimulated to induce habitual auras or attacks, particularly for medial temporal lobe epilepsy (MTL). (Chauvel et al, 1993)

E. Physiology of paired pulse depression and facilitation

A more sophisticated version SPES is provided by applying two identical electrical pulses with a brief period between them (hereafter called paired pulse electrical stimulation or PPES). The aim of paired pulse electrical stimulation (PPES) is the detection of changes in excitability resulting from the mechanism explained below. PPES is based on the principle that when two pulses are delivered one shortly after the other, the response elicited by the first or "conditioning" stimulus may influence the response elicited by the second or "test" stimulus so that the amplitude of the response of the second pulse of PPES can be: a) ***suppressed*** when the amplitude of the response to the second pulse is absent, b) ***depressed*** when the amplitude of the response to the second pulse is reduced compared to the response to the first pulse (paired pulse depression, or PPD), c) ***facilitated*** when the amplitude of the response to the second pulse is increased compared to the response to the first pulse (paired pulse facilitation, or PPF), or can show d) ***no change*** when the amplitude of the response to the second pulse is similar to the response to the first pulse.

E.1. Statistical nature of synaptic transmission

It is well established that in chemical synapses transmission is mediated by a basic unit the quantum, which corresponds to the postsynaptic response elicited from the neurotransmitters packaged in a single presynaptic vesicle. The amplitude of the postsynaptic response though is not always the same. Under certain circumstances postsynaptic response may differ from stimulus to stimulus (Kullman 2007). This fluctuation is a reflection of the various patterns of synaptic plasticity (facilitation or depression) and lies at the heart of neuronal transmission. As a result, neuronal transmission at synaptic level is an entirely stochastic process (Bliss et al 2007).

In order to better describe its probabilistic nature, statistical analysis (quantal analysis) has been used, and the following parameters/variables were defined (Bliss et al 2007, Silver et al 2003, Schneggenburger et al 2002, Zucker 1973, Zucker 1989 and Zucker and Regehr 2002): 1) The release probability *(p)* at each site; 2) the number *(n)* of independent release sites or active zones with the available vesicles ready to be released;

and 3) the quantal amplitude *(q)* defined as the amplitude of the postsynaptic response elicited by the amount of neurotransmitter contained inside a single vesicle. Any increase or decrease in the first two parameters would be presynaptic in origin as opposed to the quantal amplitude (size) which would reflect a postsynaptic change (Bliss et al 2007, Silver et al 2003).

Quantal analysis has showed that, during short-term enhancement (enhancement of the response to the second pulse compared to that of the first pulse), a modification of the number (*n*) of independent release sites or active zones clustered with vesicles ready to be released takes place, rather than a modification of the quantal amplitude. Therefore, a presynaptic mechanism was proposed for short term enhancement (Zucker and Regehr 2002).

Zucker (1973) has elucidated the physiological and anatomical context of these variables in crayfish. He showed that: 1) once a second pulse was delivered shortly after another, then the response to the second pulse was enhanced compared to the response of the first pulse; 2) during such facilitation the average number *m* of quanta available to be released would be increased and such an increase would depend on their increased release probability; and 3) the neurotransmitter would be released at discrete release sites occupied by a certain number of quanta. According to Zucker's model, there should be a limiting factor for the maximum number of quanta that can be released at a specific site. This limiting factor was defined as the restricted number of release sites. In such a case *n* would be an estimation of the number of these sites. The release probability would then depend on: 1) the probability (p2) that a neurotransmitter would be released by an invading action potential from its occupied site; and 2) the probability (p1) that this site would be refilled by another vesicle.

On the other hand, Worden and colleagues (1997) investigated the physiology underneath facilitation in the lobster neuromuscular junction. Their results showed that: 1) the average release probability (*p)* of the ready to be released vesicles and the number (*n)* of synaptic vesicles ready to be released would depend on stimulation frequency. When stimulation frequency is increased neurosecretion is enhanced (frequency facilitation) This in turn would imply that in order for the vesicles to be successfully docked and eventually fused emptying their contents in the synaptic cleft, repetitive stimulation was

needed; 2) when the quantal content was not saturated during strong facilitation over the analyzed frequency range, the release probability *p* was then saturated in lower stimulation frequencies; then any increment in the quantal content (product of the first two parameters) during high frequency stimulation would depend on the number *n* of vesicles, suggesting that probability *p* does not constitute the limiting factor for the neurotransmitter release. According to their theory: 1) the quantal content would depend on both variables *n* and *p;* 2) the factor that restricts neurotransmitter release is the rate of mobilization and demobilization; and 3) an increased rate of quantal demobilization reflected enhanced frequency facilitation.

In this extremely interesting study, Worden and colleagues also compared these two models (Worden et al. 1997, and Zucker 1973) of facilitation. According to Zucker, the maximum number of released vesicles is restricted by a finite number of release sites (the physical factor), but according to Worden and colleagues the limiting factor is the rate of mobilization and demobilization of vesicles (the non-physical factor). A second important difference would be the assumption made by Zucker that, from the moment that "non-active" zones can be recruited and activated, release probability values would be non-uniform and therefore the p-value in these "non-active" areas will be low. No such assumption was made in the stimulation-dependent mobilization model of Worden and colleagues.

E.2 Mechanisms underlying paired pulse facilitation.

E.2.1 Presynaptic mechanisms.

E.2.1.1 Intracellular residual Ca++ hypothesis

This theory was originally put forward by Katz and Miledi (1968) in their work in neuromuscular junctions of frog. They showed that if two pulses were delivered one shortly after the other, the degree of facilitation of the response elicited by the second pulse would depend on the intracellular concentration of calcium which in turn was determined: a) by the reduction of calcium concentration after the first pulse; and b) by the removal of calcium from the critical sites.

They inferred that if the inter-pulse interval was too short and the calcium concentration after the 2nd pulse was greater than the calcium concentration after the 1st pulse, the response after the 2nd pulse would be facilitated. However, for longer inter-pulse intervals, facilitation would be determined on the residual concentration of calcium after the 1st pulse.

It can be easily concluded that the role of calcium is in the centre of this theory (Zucker and Regehr 2002). Subsequent studies have confirmed this hypothesis by investigating the relationship between calcium influx and neurotransmitter release (Zucker 1989, Wu-Saggau 1994, Sakaba and Neher 2001). In particular a linear relationship between the extracellular concentration of calcium and the neurotransmitter ready for release was found (Zucker 1989, Wu and Saggau 1994, Sakaba and Neher 2001). According to Zucker and colleagues, who used microspectrophotometry to detect calcium entry at the squid giant synapse, two calcium ions were necessary for the neurotransmitter release. In other words, a 2nd power relation was detected during PPES, when an inter-pulse interval of 100msec was applied. He suggested that this could serve as a predictor for the neurotransmitter release, and therefore of the time course of the paired pulse facilitation. Wu and Saggau in their interesting study in the CA3-CA1 synapses in the hippocampus of the guinea pig corroborated the linear relationship between calcium entry and neurotransmitter release, in the 4th power though.

Sakaba and Neher, on the other hand, provided further evidence by studying the calyx of Held synapse. They determined that 3 or 4 calcium ions are necessary for neurotransmitter release. They also found that: a) the application of strong stimuli with an interval longer than 60 msec would result in the recruitment of more vesicles from the readily released pool contributing therefore to their total release; b) if the concentration of calcium entry is small after the 1st pulse it will be increased after the 2nd pulse; and c) the release rate of the synaptic vesicles is heterogeneous. There is a general agreement that three to four calcium ions bound to its sensor are necessary to trigger release (Thomson 2000).

Further insight was provided when the issue of frequency-dependent transmission of mossy fibers was compared with the transmission observed in the associational pathways in CA3 region of guinea pigs (Salin et al 1996).

Their results showed that: a) facilitation in association pathways was present at higher frequencies than that observed in mossy fibers; b) the magnitude of facilitation was found to be higher in mossy fibers (6-fold) than in the association pathways at the same frequency (0.3 Hz); c) the use of the calcium chelator EGTA blocked paired pulse facilitation and reduced frequency facilitation mainly below 0.2 Hz; d) a specific calcium/calmodulin-dependent kinase II (CAM kinase II) inhibitor (KN62) inhibited facilitation. They inferred that: a) there is a specific relationship between calcium influx and PPF; b) CAM kinase II mediates facilitation; and c) anatomical differences (number of active zones or proteins involved in vesicle release), lower release probability, and different calcium buffering would be some explanations of the different magnitude of facilitation observed between mossy fibers and associational pathways.

A very interesting study was performed by Caillard and colleagues (2000) who shed some light to the role of the Ca –binding protein parvalbumin in short-term plasticity. They tested this hypothesis in GABAergic synapses in the Purkinje cells of mice with and mice without the gene for parvalbumin. They showed that: a) the mice without the gene developed PPF and the mice with the gene developed PPD; and b) the addition of the calcium chelator agent EGTA in mice without the gene reinstated PPD. They suggested that parvalbumin accelerates the initial decay rate of the calcium influx reducing the residual calcium concentration with consequent paired pulse depression.

E.2.1.2 Other mechanisms

Although the main proposed model would be the residual Ca++ theory there are other presynaptic mechanisms that might contribute to paired pulse facilitation. The action of the protein kinases could be one of them (Yawo 1999, Hilfiker and Augustine 1999).

Yawo investigated the involvement of protein kinase C (PKC) in neurotransmitter release using the chick ciliary ganglion. He showed that: a) PMA, a phorbol ester, increased EPSPs with no effect on the nichotinic acetylcholine (Ach) receptors; b) PMA reduced the PPF ratio while 2 protein kinase C inhibitors blocked its effect; c) the effect of PMA was less when the extracellular concentration of calcium was higher; d) PMA did not affect the intraterminal calcium concentration either at rest or after an invading action potential; and e) it did not affect the recovery state after an induced titanic depression.

The results indicated the involvement of PKC in enhancing the calcium sensitivity process of fusion probability.

Synaptotagmin is a synaptic vesicle protein, which serves as a calcium sensor for triggering exocytosis (Chapman et al 1995). A discrepancy in the expression of these calcium detectors could result in an increase in the probability of exocytosis (Geppert and Sudhoff 1998).

Moreover the distribution and properties of calcium channels, as well as the shape of the invading action potential, may influence the probability of release of the vesicle (Thompson 2000).

E.2.2 Postsynaptic mechanisms.

In dendritic coincidence detection under certain conditions, antidromic stimulations interact with appropriately timed EPSPs only for the amplitude of the distal dendrites to be amplified (Stuart and Hauser 2001, Larcum et al 1999). Stuart and Hausser (2001) showed that the increase in amplitude was due to the activation of sodium channels because tetrodoxin (TTX sodium channel blocker): a) blocked the amplification of small action potentials by EPSPs; and b) it did not affect the amplitude of only the small action potentials at the dendrites. Further insight was provided by the interesting work of Larcum et al. (1999). They showed that a sub-threshold EPSP and an antidromic stimulation properly timed resulted in an increase of Ca++ influx which in turn lowered the threshold and generated a burst of action potentials in the soma.

An interplay between AMPA and NMDA receptors could shift the balance towards PPF (Bouteiller et al 2008, Bouteiller 2010). Bouteiller et al (2010), with the help of a computational modelling system found that AMPA receptors exhibited PPD at small interpulse intervals due to desensitization while NMDA receptors contributed to facilitation due to the summation effect. They suggested that the number of postsynaptic receptors expressed as a ratio (AMPA receptors / NMDA receptors) is crucial for the development of paired pulse facilitation or depression.

Polyamine dependent facilitation appears to be another mechanism involved in PPF (Rozov and Burnashev 1999, Bagal et al 2005). They studied *in vitro* the neocortical pyramidal neurons of the rat and they showed that during high frequency stimulation the removal of the polyamine block from polyamine sensitive AMPA receptors resulted in facilitation in the context of desensitization or presynaptic depression.

E.3. Mechanisms underlying paired pulse depression

E.3.1. Presynaptic level

Depletion of neurotransmitter mechanism

This theory was first proposed by Liley and North in 1953 in an effort to elucidate the effects of tetanic stimulation (TS) in the neuromuscular junction of rats under curare. They observed that: a) the higher the frequency of TS (keeping duration of TS constant) the higher the post-tetanic potentiation (PTP); b) on very low or very high frequencies PTP was not observed; c) the higher the duration of TS the higher the PTP and the time to reach such a potentiation with a slower decay course; d) an increase in the size of the end-plate potentials (e.p.p's) with a time course different than the time course of PTP; e) a decrement in the concentration of the extracellular potassium (K) would result in an increase of the extend of PTP; f) repetitive direct TS of the muscle produced no increment in the size of the e.p.p. by following stimuli; g) the degree of PTP was augmented by augmenting curare's concentration; and h) no effect on the PTP was seen with addition of anticholinesterases. According to the authors, the increase of the size of the e.p.p observed during PTP was due to the amount of neurotransmitter released. The initial decline and subsequent enhanced e.p.p observed after tetanus would correspond respectively to a decrease and an increase of the amount of neurotransmitter released. This in turn would be determined by the amount of neurotransmitter at the nerve terminals (concentration factor) and the degree of readiness of the neurotransmitter for release (readiness factor). The most likely mechanism for the facilitated effect noted after tetanus would be a reduction of the intracellular rather than an increase of the extracellular concentration of potassium as in the latter case the potassium would be dispersed in the surrounding areas.

However, these results were interpreted under two assumptions: a) that during TS, the fraction of the releasable amount of neurotransmitter would be constant after each invading potential; and b) that the releasable pool of vesicles is slowly replenished reaching extremely low levels. However, the above features were challenged when it was found that the observed depression was less than predicted from this model (Zucker 1989). Therefore, this theory was later advanced and refined. More specifically Betz (1970) in his experiments at the neuromuscular junction of frog observed that the amount of neurotransmitter released, and consequently the extent of depression, would change according to the number of stimulations and the calcium concentration. Moreover depression was detected after action potentials were abolished by tetrodoxin.
These results argued against the hypothesis that one of the possible mechanisms of depression would be the block of the propagation of the action potential in one of the terminal branches, and corroborated in favour of the "depletion theory" as proposed by Liley and North. They also inferred that during depression the probability *p* of the amount of neurotransmitter available to be released is reduced as well as the amount of neurotransmitter contained in the vesicles of the releasable pool.

Further light was shed by the use of the capacitance technique (Gersdorff and Matthews 1999, Gersdorff and Borst 2002). Gersdorff and Matthews have used this rather sophisticated technique to study the kinetics of exocytosis and endocytosis of a synaptic vesicle. This technique was based on the fact that whenever a synaptic vesicle was fused with the plasma membrane capacitance is increased, and whenever the synaptic vesicle was removed from the membrane capacitance was reduced. Therefore they simply measured in the bipolar cells of goldfish the specific capacitance of the active zones ("ribbons") where synaptic vesicles are clustered. The following interesting results were shown: a) After an invading activation potential capacitance is increased; b) When a second potential is applied, the second increase in the capacitance noted is smaller than the first; c) The recovery from paired pulse depression in bipolar cells is around 8 sec; d) Saturation was noticed after a limit with no change in the capacitance responses; e) They estimated that in synaptic ribbons the number of synaptic vesicles would be about 5700. They concluded that: a) depression could not be explained by inactivation of calcium channels and synaptic vesicle depletion was the most probable explanation; b) saturation

could be interpreted as an exhaustion of readily releasable pool c) synaptic vesicle would be readily released.

A different approach was used by Koenig and colleagues (1983) who studied the synaptic transmission in a mutant of the Drosophila Melanogaster. It was seen that in elevated temperature (29 Celcius), EPSPs were reduced and vesicle depletion was seen. When temperature was lowered, conditions were reversed.

E.3.2. Postsynaptic mechanisms

Desensitization and saturation

Saturation occurs if, during the second pulse, postsynaptic receptors are still bound to the neurotransmitter released by the first pulse. In the extreme case that all receptors are bound to neurotransmitter, no post-synaptic response is induced (Yang-Friedman 2008, Kirischuk et al 2002). On the other hand during desensitization, a functional change of the receptor gating occurs from an open state to a fully bound non-conductive state so that no current is allowed during a second stimulation (Yang-Friedman 2008, Kirischuk et al 2002).

Yang and Friedman studied the mechanisms of synaptic depression in the endbulb of Held synapse in the anteroventral cochlear nucleus in mice. They found that: a) depression of both the AMPA and NMDA currents is described by two phases of recovery slow and fast; b) the slow component of AMPA current and the slow component of NMDA current have similar kinetics implying similar underlying mechanisms; on the contrary their fast components display totally different time course; c) in the presence of cyclothiazide (CTZ) and aniracetam the decay phase of the AMPA EPSC was prolonged and the PPR was increased (short IPIs) implying desensitization; d) CTZ produced no effect on the depression of the NMDA EPSC; e) the PPR of the NMDA EPSC between controls and CTZ is similar; and f) the use of a low affinity NMDA receptor antagonist diminished the fast but not the slow component of this current with saturation the entailed mechanism .

A remarkable study was performed by Kirischuk and colleagues (2002). They investigated the mechanisms underlying depression at the level of a single GABAergic bouton in the rat, specifically at the two kinetically different components of the recovery of depression, paired pulse depression slow (PPD_{slow}) and paired pulse depression fast (PPD_{fast}). They first showed that activation of $GABA_B$ receptors could not account for depression because a specific $GABA_B$ antagonist (CGP55845A) did not influence PPD. They also provided evidence that PPD_{slow} had the largest impact on depression. The implication of this would be that the mechanisms underlying PPD_{slow} would probably have a major influence on the mechanisms underlying depression.

They further looked into the mechanisms underlying PPD_{slow}, and reported the following results: a) EGTA did not change the mean amplitude of the first inhibitory postsynaptic current (IPSC); b) clonazepam (CLZ) a benzodiazepine that increases $GABA_A$ receptor occupancy and affininity would not alter the paired pulse ratio (PPR); c) a relation between PPR and the failure rate was not proved at different concentrations; and d) a negative correlation was found between the first and the second inhibitory postsynaptic potential. From the above they concluded that any postsynaptic mechanism (desensitization) or the increased rate of clearance of presynaptic residual calcium would be unlikely to constitute the basis of PPD. They also inferred that a release-independent inhibition of exocytosis would be the most probable cause for PPD_{slow}.

They showed that there are two mechanisms involved in PPD_{fast}: a presynaptic and a postsynaptic mechanism. The presynaptic mechanism was found to be related to the extracellular calcium concentration as PPD_{fast} was diminished in the presence of the calcium chelator EGTA. However as the replenishment of calcium by diffusion in the extracellular medium is quite fast (10 msec) depletion of calcium be unlikely to constitute an explanation for PPD which was seen at longer IPIs (100 msec). They suggested that calcium-dependent inactivation of voltage gated calcium channels would be the most plausible candidate. A postsynaptic element (desensitization) was also proposed for PPD_{fast} which was enhanced in the presence of clonazepam. On the other hand the amplitude of inhibitory postsynaptic currents (IPSCs) was depressed after the addition of the low affinity and rapidly equilibrating $GABA_A$ receptor antagonist (TPMPA).

F. Paired pulse electrical stimulation in epilepsy

Changes in excitability resulting from the mechanisms detailed above can be responsible for epileptic seizures, and both PPF and PPD have been described in epilepsy.

F.1. Animal studies

Increased inhibition

Increased paired pulse depression has been reported in a number of studies in vitro and in vivo in rat (Tuff 1983 a, Tuff 1983 b, King et al 1985, De Jonge and Racine 1987, Stringer and Lothman 1989, Davies et al 1990, Sloviter 1991a, Zhao and Leung 1992, Milgram et al 1995, and Haas et al 1996).

The aim of Stringer and Lothman's interesting study (1989) was to confirm if repeated seizures would produce any changes of inhibition. For this reason they followed the paired pulse electrical stimulation protocol before and after multiple seizures, by stimulating the CA3 region of the left hippocampus and the right angular bundle and recording from the dentate gyrus. A moderate increase in paired-pulse depression was seen after 36 seizures and a further increase after 72 seizures

King (1985) et al. confirmed the above findings using the kindling model of epilepsy in rats. They stimulated the lateral entorhinal area and recorded from the dentate gyrus. They observed that: a) the current intensity required to evoke a standard-size population spike was elevated; b) paired pulse inhibition was greater in the kindled slices of the dentate gyrus; c) no differences in excitatory postsynaptic potentials (EPSPs) between control and kindled slices were noted; d) paired pulse inhibition was reduced by GABA antagonists. The authors concluded that the above findings express enhanced synaptic inhibition rather than decreased excitability. Considering also that paired pulse inhibition disappeared 28 days after kindling the theory of the dentate gyrus playing a protective role to seizure propagation through the entorhinal cortex was suggested.

De Jonge and Racine (1987) used the kindling model of epilepsy in rats to show the time course of paired pulse depression after stimulation of the perforant path and dentate gyrus. An early phase was seen after the 1st kindling stimulation while a later phase after the completion of 10 stimulations. When tetanization stopped the late component decayed faster than the early one.

Further insight about the role of increased inhibition with regard to the pathogenesis of human partial epilepsy was provided by Haas et al. (1996) who used the kainic acid (KA) model of epilepsy in order to provoke status epilepticus in rats. They observed that: a) bicuculline blocked early inhibition, showing that both postsynaptic receptors ($GABA_A$ and $GABA_B$) are involved in enhanced inhibition; b) both $GABA_A$ and $GABA_B$ responses were increased in granule cells by a specific $GABA_B$ antagonist (CGP 35348), implying a rather presynaptic involvement of CGP 35348; and c) early disinhibition, which was reduced after seizures, was blocked by the $GABA_B$ antagonist CGP 35348 in controls and to a minor degree to KA-treated rats as opposed to the late disinhibition which remained intact after seizures.

They concluded that a down-regulation of $GABA_B$ presynaptic receptors, normally suppressing GABA release, is partly responsible for the enhanced dentate inhibition observed in both the early ($GABA_A$ receptor-mediated inhibition) and late ($GABA_B$ receptor-mediated inhibition) phase. It was the last finding that proved that a downregulation rather than a completely loss of $GABA_B$ presynaptic receptors, is in order. Therefore and according to their opinion, increased dentate inhibition would reflect a compensatory mechanism for the hippocampal hyperexcitability.

Reduced inhibition

Absence of paired pulse depression was confirmed with different model of epileptogenesis (Kamphuis et al 1988, Kapur et al 1989, Sloviter 1991, Shirasaka and Wasterlain 1994, Emori et al 1997, Ikeda-Douglas et al 1998, Fueta et al 1998, Naylor 2002, Naylor and Wasterlain 2005, Schmidt et al 2006).

Kapur et al (1989) used PPES in anesthetized rats in the contralateral CA3 region for the activation of the contralateral CA1 area through the hippocampal commissure. They observed that: a) repetitive seizures caused significant attenuation of the early phase of paired pulse depression; b) trains of electrical stimuli produced a progressive lengthening of afterdischarges; and c) GABA agonist muscimol enhanced and bicuculline reduced paired pulse depression (early phase). They concluded that PPD reflects in hippocampus the potency of GABAergic inhibition and threshold.

The kindling model of epilepsy was used by Kamphuis et al (1988) and PPES was applied in the Shaffer collaterals in rats recording from CA1 pyramidal cells in order to study the electrophysiological changes involved. A progressive disinhibition was noted which eventually led to excitation due to the reduced paired pulse inhibition and threshold of the pyramidal cells.

Further evidence of loss of inhibition was provided by Naylor (2002). He stimulated the perforant pathway in rats where the status epilepticus model of epilepsy was used. He noticed that brief stimulations resulted in loss of inhibition which was interpreted as an early transition to status. Consistent with this were his findings of reduced benzodiazepine receptor sensitivity affecting GABAergic function. In an analogous study he and Wasterlain (2005) corroborated the previous evidence of loss of the early phase of inhibition after brief paired stimuli were applied in the perforant pathway of rats. They also observed that bicuculline caused similar loss of inhibition supporting their previous conclusion.

Shirasaka and Wasterlain (1994) investigated the chronic changes in kindled rats using the focal status epilepticus model. Stimulation of the perforant pathway showed that for short (10-100msec) and long (200-1000 msec) interpulse intervals, PPD was lost after 30 minutes. It returned at approximately the same levels after 4 weeks for short interpulse intervals, but not for longer interpulse intervals where after 4 weeks a significant difference was seen with control animals.

Ikeda-Douglas et al (1998) used the kainic acid model of epilepsy in rats where the perforant pathway was stimulated with PPES at short (20-30 msec) intermediate (45-90 msec) and long (200-300 msec) interpulse intervals.

Paired pulse suppression of the spikes was noted in the control group at the short (early phase) and long (late phase) interpulse intervals. At intermediate interpulse intervals an intermediate facilitation was seen. After injection of kainic acid, suppression was lost only for short interpulse intervals. The authors concluded that: a) the early and late phase most likely correspond to inhibition; and b) this early loss of inhibition could be interpreted in the context of a reduced $GABA_A$ or an increased $GABA_B$ function or even a combination of both.

F.2. Human studies

Uruno et al (1995) studied the phenomenon of inhibition *in vitro* in patients with intractable partial epilepsy. Perforant pathway was stimulated with paired pulses while recording from the granule layer of dentate gyrus. Two groups were seen according to the magnitude of the highest paired pulse ratio defined as the population spike amplitude of the second response (PS2) to the population spike amplitude of the first response (PS1): a) a strong group (PS2/PS1=0.12), and b) a weak group (PS2/PS1=0.68). The strong group, as opposed to the weak group, is independent on stimulus intensity. It also showed significant reduction of PPD, after pharmacological blockade of bicuculline and baclofen, and occasionally even paired pulse facilitation. These findings were consistent with the presence of: a) increased inhibition in the strong group; b) reduction of feedback inhibition and, in some cases paired pulse facilitation, after the use of pharmacological agents.

Increased paired pulse depression was observed in human epileptic hippocampus. Wilson et al. (1998) studied twenty patients with medically intractable temporal lobe epilepsy who were candidates for anterior temporal lobe resection. The authors compared the hippocampal pathways between the epileptogenic and the contralateral non-epileptogenic hippocampus and found that: a) paired pulse suppression was greater in the epileptogenic side; b) paired pulse suppression was greater in the epileptogenic rather than the non-epileptogenic perforant path; and c) paired pulse depression was greater in the epileptogenic hippocampus following stimulation of the perforant path, rather than stimulation of intrinsic associational pathways. The authors interpreted these findings as evidence for synaptic reorganization following the loss of the principal neurons.

From the above animal and human studies, it can be concluded that PPES has provided evidence in favour of both mechanisms of epileptogenesis: a) increased excitation or reduced inhibition (facilitation); and b) increased inhibition (depression).

G. Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a safe non-invasive method which can be used to investigate: a) corticospinal tract function in normal and brain damaged patients (Yeomans Oxford Univerity Press 1989, Sole et al 1992, Kujirai et al 1993, Nakamura et al 1997, Ziemann et al 1998, Ilic et al 2002, Tassinary et al 2003, and Di Lazzaro et al 1999a, 2000b, 2002c and 2008d); and b) the mechanisms of epileptogenesis (Badawy et al 2007a, 2009b, 2012c, Wright et al 2006) and identify seizure onset areas (Valentin et al 2008).

During TMS, the rapidly alternating magnetic field induced by a large current, induces a smaller current (Ziemann et al 1998, Richardson and da Silva 2011). The stimulation is usually applied in the primary motor cortex at the area of the target muscle (usually 1st dorsal interosseus) and the recording electrode over the target muscle records the electromyogram (EMG) response to stimulation (Richardson and da Silva 2011). The EMG response after the stimulation is often called the motor evoked potential (MEP).
Once the motor cortex is stimulated, a series of waveforms can be recorded if a recording electrode is also placed in the medulla or the spinal cord. The earliest waveform refers to the direct ("D") activation of the axons of the fast pyramidal neurons (Di Lazzaro et al 2008). The following waves refer to the indirect ("I") transynaptic activation of pyramidal tract neurons (Di Lazzaro et al 2008).

For the investigation of the excitatory and inhibitory functions the technique most commonly used is the paired pulse TMS, during which a conditioned stimulus is applied, followed by a test stimulus. Paired pulse TMS is based on the fact that the size of the MEP elicited by the test stimulus is influenced by the conditioned stimulus. There are two approaches in paired pulse TMS.

First, identical suprathreshold pulses of the same intensity can be used as conditioned and test stimuli. Second, subthreshold conditioned and suprathreshold test stimuli can be applied.

Paired pulse TMS studies have used various interstimulus intervals (1msec-400 msec). Both intracortical facilitation and inhibition (short and long-interval intracortical inhibition) and have shown. Kujirai et al showed that with short interstimulus intervals (1-5 msec) and subthreshold conditioned stimuli have shown the late I-waves are reduced in size in normal subjects (Kujirai et al 1993). This short-interval intracortical inhibition (SICI) was considered by the authors to occur at a cortical level because no MEP was elicited during the subthreshold conditioned stimuli.

Inhibition has also been observed with longer interstimulus intervals (Long Intracortical Inhibition or LICI)**.** Suprathreshold conditioned and test stimuli have shown prominent inhibition of the late I-waves at 100-200 msec interstimulus intervals in five non-epileptic patients (three with aortic aneurysm and two with cervical radiculopathy) (Nakamura et al 1997). As the H-reflex has recovered during this period, it was thought that cortical mechanisms were the basis of long intracortical inhibition. Di Lazzaro et al confirmed the above findings of inhibition when an inter-stimulus interval of 100 msec and 150 msec was used in four patients (three for treatment of lumbar-sacral pain and one patient with vascular parkinsonism) (Di Lazzaro et al 2002). However, the later components of the indirect waves (I2-I4) were slightly increased at 50 msec interstimulus interval and the MEP was reduced implying that MEP inhibition takes place at a subcortical (probably the spinal cord) level.

Intracortical facilitation has also been seen with short interstimulus intervals of up to 5 msec with intensities at or above the resting motor threshold for both stimuli (Ziemann et al 1998, Ilic et al 2002). Motor evoked potentials were also facilitated when subthreshold conditioned stimuli were used with longer interstimulus intervals of 10-15 msec (Kujirai et al 1993).

A very interesting study was performed in 23 patients with idiopathic generalized epilepsy and 35 patients with focal epilepsy (Badawy et al 2009).

The authors studied changes in cortical excitability occurring in the peri-ictal period. The main findings consisted of pre-ictal increase in excitability (facilitation) and post-ictal inhibition. These findings were seen bilaterally in patients with idiopathic generalized epilepsy and in patients with focal epilepsy with secondarily generalized seizures. Results in patients with focal epilepsy without secondarily generalized seizures were slightly different. During the pre-ictal period, facilitation was seen in the hemisphere ipsilateral to the focus. However, inhibition was mainly seen in the contralateral hemisphere. In the post-ictal period, inhibition was noted for short (2-15 msec interstimulus interval) and long (50-400 msec) interstimulus intervals with an intermediate period of facilitation. The authors concluded that facilitation represents the spread of seizures independently on the aetiology, whereas inhibition can be considered as a protective mechanism which when failed favors the spread of seizures.

From the above studies, it can be concluded that TMS has provided evidence for both, increased excitation or reduced inhibition, as mechanisms involved in epileptogenesis.

HYPOTHESES AND SPECIFIC AIMS

Hypotheses

The following hypotheses were tested with regard to responses to paired pulse electrical stimulation applied via intracranial recordings:

1. Suppression, depression or facilitation induced by PPES is related to the epileptogenic potential of the underlying cortex, i.e. they are more frequently observed in the seizure onset area, compared to other cortical regions.
2. The presence of suppression, depression or facilitation could be used to predict seizure control after surgery, i.e. removal of the cortex showing suppression, depression or facilitation is associated with better seizure control after surgery.

Specific aims

The following specific aims were set out in order to test the above hypotheses:

1) To compare averaged responses to SPES and PPES (see methods) in order to identify regions showing suppression, depression or facilitation.
2) To compare the proportion of patients showing suppression, depression or facilitation, in non-epileptogenic and in epileptogenic lobe/areas defined according to the location of seizure onset on intracranial EEG.

STUDY DESIGN AND OUTLINE

In order to study the hypotheses stated in the previous paragraphs, this study has been carried out in four stages:

1) Pilot studies were initially carried out in a small number of patients in order to:
    a) Chose the interpulse interval for PPES;
    b) Estimate the effects of the jitter induced on the recognition of the stimulus artefact by aliasing of the stimulus artefact by the sampling rate used;
    c) Estimate the effects of the artefact correction method used by the ASA analysis software (see Methods); and
    d) Choice of response type (depression, suppression or facilitation) that may be best correlated with epileptogenesis.

2) Visual analysis of all 79 patients included. Only analysis of suppression was attempted visually, as this an on-off response type (i.e. it could be visually identified as either present or not present) that could be reliably identified visually.

3) For analysis of facilitation and depression, a computer programme was implemented, which was initially tested on suppression.

4) Analysis of depression, facilitation and suppression, identified automatically.

METHODS

METHODS

A. DATA COLLECTION

A.1. Subjects

The study included patients with medically intractable focal epilepsy assessed with intracranial electrodes between 2000 and 2010 for resective surgery as a treatment of their epilepsy. As non-invasive tests failed to reliably localize the epileptogenic zone, they were admitted at King's College Hospital for video telemetry with intracranial recordings.

Patients were informed about the nature of the research and gave full consent according to the Declaration of Helsinki. The research project was initially approved by the ethical committee of King's College Hospital (reference number 99-017). During the course of this research, it was realised that PPES could sometimes induce abnormal responses where SPES of similar intensity could not, and consequently PPES with 200 ms interstimulus interval was added to the clinical protocol. Both SPES and PPES are now part of the clinical protocol for presurgical assessment of patients with epilepsy with intracranial electrodes. Correlation of SPES and PPES features with surgical outcome has been approved by the Neuroscience Audit Committee at King's College Hospital as part of an audit study.

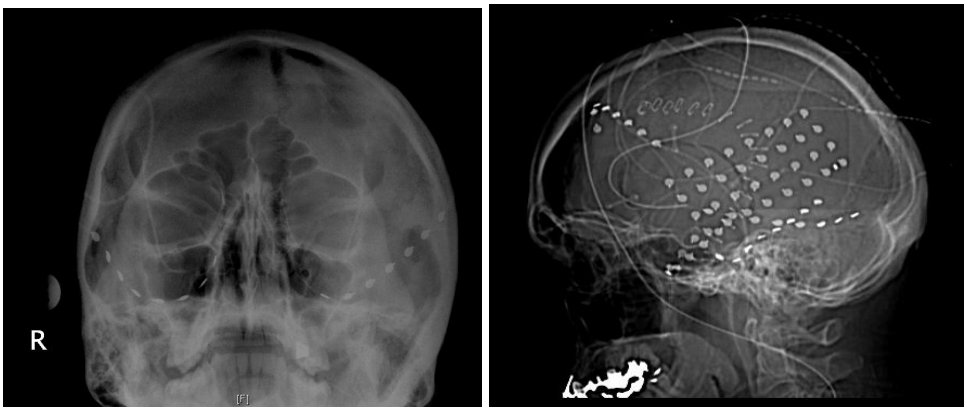
A.2. Electrode placement

The intracranial electrodes (AdTech Medical Instruments Corporation WI, USA) used in this study were depth (intra-cerebral) or subdural electrodes. Subdural electrodes were strips or mats.

The type, number and location of electrodes implanted were determined by the suspected location of the epileptogenic zone in each patient according to clinical history, neuroimaging, neuropsychology and scalp EEG recordings. The selection criteria and implantation procedure were as described previously (Alarcon et al. 2009**)**. The implantation was performed by the neurosurgical team at King's College Hospital, independently from this study.

A.2.1. Subdural electrodes

Strips of electrodes consisted of 4 or 8 platinum disc electrodes arranged in a single row and embedded in a 0.7 mm thick non-conductive material (polyurethane) at intervals of 10 mm from centre to centre. Mats of electrodes consisted of 12, 32 and 64 platinum electrodes, arranged in rows of 4, 6 or 8 electrodes separated by a distance of 10 mm from centre to centre.



A ***B***

Figure 1. A. Example of 8-contact bitemporal strips. B. Example of a 32 contact left temporal mat with left fronto-temporal strips.

A.2.2. Intracerebral depth electrodes

Bundles of depth electrodes consisted of 10 cylindrical 2.3 mm long platinum electrodes, with a spacing of 5 mm between centres of adjacent electrodes in the same bundle. The electrodes were implanted stereotactically under general anaesthesia and MRI guidance (figures 2 and 3).

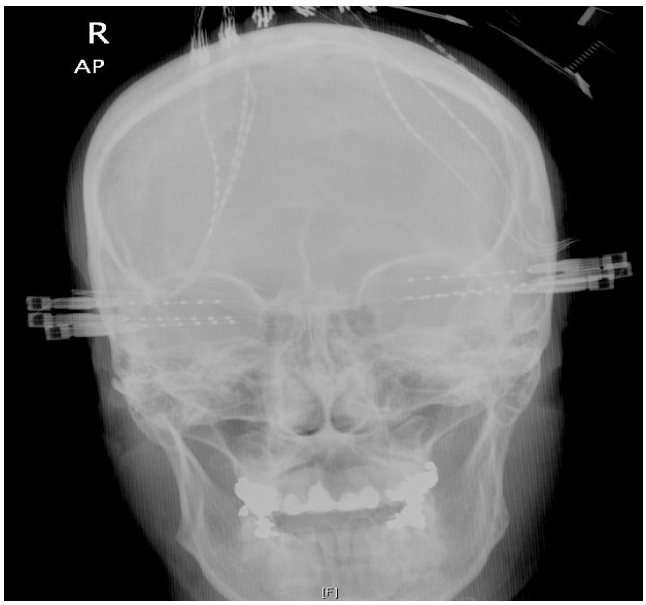


Figure 2. Example of bitemporal depth electrodes with the tips in medial temporal structures.

A.3. Telemetry EEG recordings and determination of ictal onset according to clinical criteria

Intracranial EEG recordings started 24–48 hours after surgical implantation, after patients had recovered from electrode implantation. Video-telemetry included up to 64 EEG recording channels. One of the two systems was used for data acquisition: a Telefactor Beehive-Beekeeper (Astro-Med, West Warwick, RI, USA) system or a Nervus Medelec System (Medelec, Oxford Instruments, Witney, UK).

Data acquired with a Telefactor system were digitized at 200 Hz and band pass filtered (high-pass cut off frequency at 0.3 Hz and low-pass cut off frequency at 70 Hz).
The amplifier's input range was 2mV and data were digitized with a 12 bit analogue-to-digital converter (amplitude resolution 0.488 μV). Data acquired with a Medelec system were digitized at 256 Hz, bass-pass filtered (0.3 Hz – 70 Hz), the input range was at 10 mV and data were digitized with a 22 bit analogue-to-digital converter (amplitude resolution 0.153 μV).

The sampling frequencies allowed a time resolution of 4-5 msec, which was adequate for the duration, and latency of the observed responses (in the order of tens or hundreds of milliseconds). Data were recorded as common reference with the reference electrode applied on the scalp, between Cz and Fz, and displayed in a variety of EEG montages.

Ictal onset was identified independently by two accredited electroencephalographers. The ictal EEG patterns used to identify seizure onset consisted of regular spikes, rhythmic sharp waves, spike-and-slow wave complexes, sharp-and-slow wave complexes, regular theta or delta activity, sharpened theta or delta activity or low-amplitude high-frequency activity in the beta range. During data collection, pre-processing, processing and most analysis, I was blind to seizure onset zone and clinical information from the patients studied.
Depending on the number of electrodes involved at seizure onset, seizures were classified as focal if seizure onset involved three contiguous contacts in the same lobe, regional if seizure onset involved more than three contiguous contacts in the same lobe, widespread if seizure onset involved two contiguous lobes on the same hemisphere and bilateral if seizure onset involved both hemispheres.

Consequently patients were classified according to seizure onset type as: a) Focal, if all seizures were focal arising from the same lobe; b) Regional, if all seizures were regional arising from the same lobe; c) Focal and regional, if patients showed both focal and regional seizure onset arising from the same lobe; d) independent seizure onset if patients had focal and/or regional seizures arising from two different lobes independently, e) widespread onset if patients had only widespread seizures and f) Bilateral, if seizures showed bilateral onset.

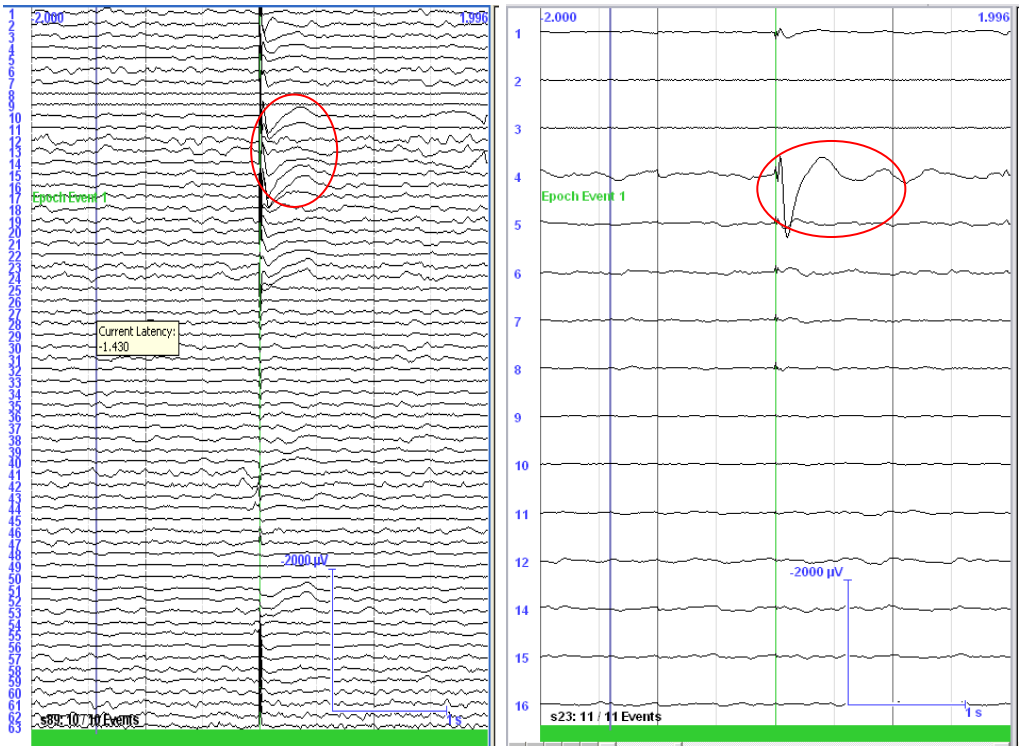
+ *Electrical stimulation*

Data collection for SPES and PPES was carried out at the patient's bedside. It took between 1.5 and 4 hours depending on the number of the implanted electrodes. Sometimes the procedure might be broken up into 2 sessions in order to minimise disruption to the clinical routines. Electrical stimulation was carried out between adjacent electrodes using a constant-current neurostimulator (Medelec ST10 Sensor, Oxford Instruments, Old Woking, UK).

The initial method used to carry out SPES has been described elsewhere (Valentin et al 2002) and this is the protocol largely used in the present study. For each pair of electrodes tested, SPES responses were recorded to 10 single pulses of one polarity and 10 single pulses of the opposite polarity. PPES was performed with 5-10 paired pulses in one polarity followed by 5-10 paired pulses in the opposite polarity. A set of electrical stimulations carried out with identical stimulation parameters in each patient will be called a "stimulation trial". For each pair of electrodes, the first trial of SPES and the second trial of PPES had the same polarity (first polarity). Similarly, the second trial of SPES and the first trial of PPES had the same polarity (second polarity). Electrical stimulation and data recording for the first 24 patients (1999-2001) were carried out before my arrival to the Department by my first and third supervisors (Drs Gonzalo Alarcón and Antonio Valentín). Stimulation and data recording for the remaining 55 patients (2009-2010) were carried out by me jointly with Dr Antonio Valentín. Pulses of 1 msec duration were applied every 10-5 sec with a current intensity of 4-8 mA (4 mA being the most commonly used current intensity) (fig 4A). Stimulation was applied between adjacent electrodes in contact with grey matter. With the intensity used, EEG responses were evoked at regions close or connected to the stimulated regions, and sometimes their duration would often exceed the duration of the interstimulus interval (usually 200 msec) (figure 3). For this reason the amplitude of the SPES responses (instead of the amplitude of the response elicited from the first pulse of the PPES) was compared with the amplitude of the response elicited from the second pulse of the paired pulse. The technique is safe (Valentin et al 2002 and Valentin et al 2005), with minimal risk of inducing seizures. If facial tingling, twitching or pain was induced by stimulation, intensity was reduced. Each element in a stimulation trial consisted of either one pulse (single pulse electrical stimulation or SPES, figure 4A) or two pulses (paired pulse

electrical stimulation or PPES, figure 4B). Monophasic pulses and bipolar stimulation between contiguous electrodes were chosen for single and paired pulse stimulation in order to increase the localizing accuracy of electrical stimulation (Jaykar 1993).

According to the standard assumptions for extracellular stimulation in Clinical Neurophysiology, it was assumed that neuronal stimulation occurred mainly at the cathode (additional extracellular negative charges will decrease membrane voltage gradient, as the inside is negatively charged at rest, thus inducing depolarisation). Consequently, for each pair of adjacent electrodes, two stimulation trials with opposite polarity were carried out in order to stimulate cortex close to each implanted electrode. During each trial, recording from the electrodes used for stimulation was not possible.



A ***B***

Figure 3. Two examples of 10 averaged responses to single pulse electrical stimulation (SPES) in a frontal (3A) and a temporal (3B) patient. The sharp vertical deflexion in the middle of both traces is the stimulation artefact. Note that the duration of the responses (red circle channels) in 5A exceeds the 300 msec and in 5B the 400 msec.

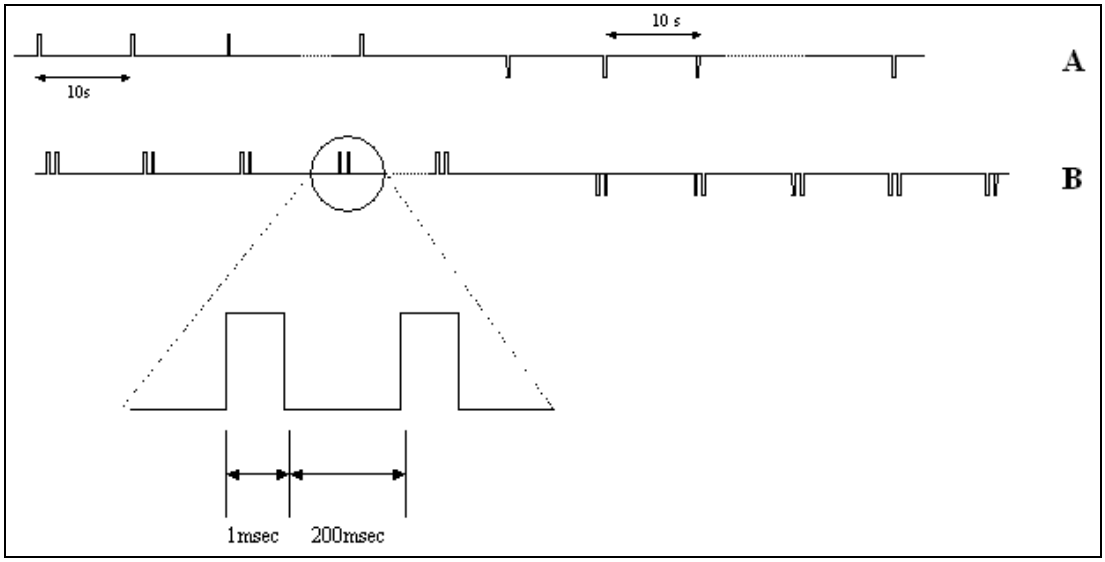


Figure 4. Representation of the timescale of single pulse electrical stimulation (SPES) and paired pulse electrical stimulation (PPES). A) SPES was performed with single pulses of 1 msec duration delivered every 10 seconds. B) During PPES, paired pulses of 1 msec duration were delivered at intervals of 10 sec with an inter-stimulus interval duration 200 msec.

B. DATA PRE-PROCESSING

Pre-processing includes all the steps required to perform the averaging and subtraction of responses described in the next section (C. Processing of data.). Two different software systems were used depending on the EEG system used for recording. Records obtained with the Telefactor system (24 patients) were pre-processed and processed with in-house QuickBasic based software (Alarcón et al., 1997). Data recorded with the Nervus system (55 patients) were pre-processed and processed with the ASA system (ANT, Advanced-Neuro-Technology, ASA, Netherlands).

With both systems, the first step for pre-processing was the identification of the stimulation artefacts of SPES and PPES to be used for synchronising events during averaging of responses within the same stimulation trial (i.e. all responses induced by identical stimuli).

Q-basic system: This software includes automatic recognition of spikes and artefacts based on detecting zero crossings and calculating a spike factor with a combination of parameters. I was not involved in developing this software, which was designed by my supervisor and co-workers well before I arrived in the Department. The details are explained elsewhere (Alarcón et al., 1997; Martin-Miguel et al, 2011). The software was modified to allow selective averaging of specific patterns after ranking detections according to values of spike factor. As stimulation artefact detections show the largest spike factors, they are identified as such, subsequently displayed and manually accepted for averaging (Martin-Miguel et al, 2011). Averaging is synchronised on the peak of the stimulation artefact.

ASA system: with the ASA system, pre-processing comprises two stages:

a) *Identification of SPES and PPES electrical stimulation artefacts***.** The ASA system can automatically identify amplitudes that pass a particular amplitude threshold (threshold level) in a specific channel (trigger channel). Therefore, before automatic recognition of stimulation artefact, the best threshold level and trigger channel had to be chosen for the software to identify the artefacts while avoiding false positive and false negative detections. Several combinations of parameters were tried before the optimal threshold level and trigger channel were found for each recording. Once detected, the threshold level and trigger channels were fed into the software in order to automatically identify stimulation artefacts from SPES and PPES.

b) *Identification of different stimulation parameters***.** The stimulation artefacts of SPES and PPES were then manually labelled in order to separate trials corresponding to different stimulation conditions. This involves labelling the detected stimulation artefacts corresponding to identical stimulation parameters (i.e. with similar polarity, stimulation site, whether single or paired pulse) (figure 5). This allows averaging of responses from the same stimulation trials (figures 6 and 7), and subtraction of the appropriate responses during processing of data as described in the following section (figure 8).

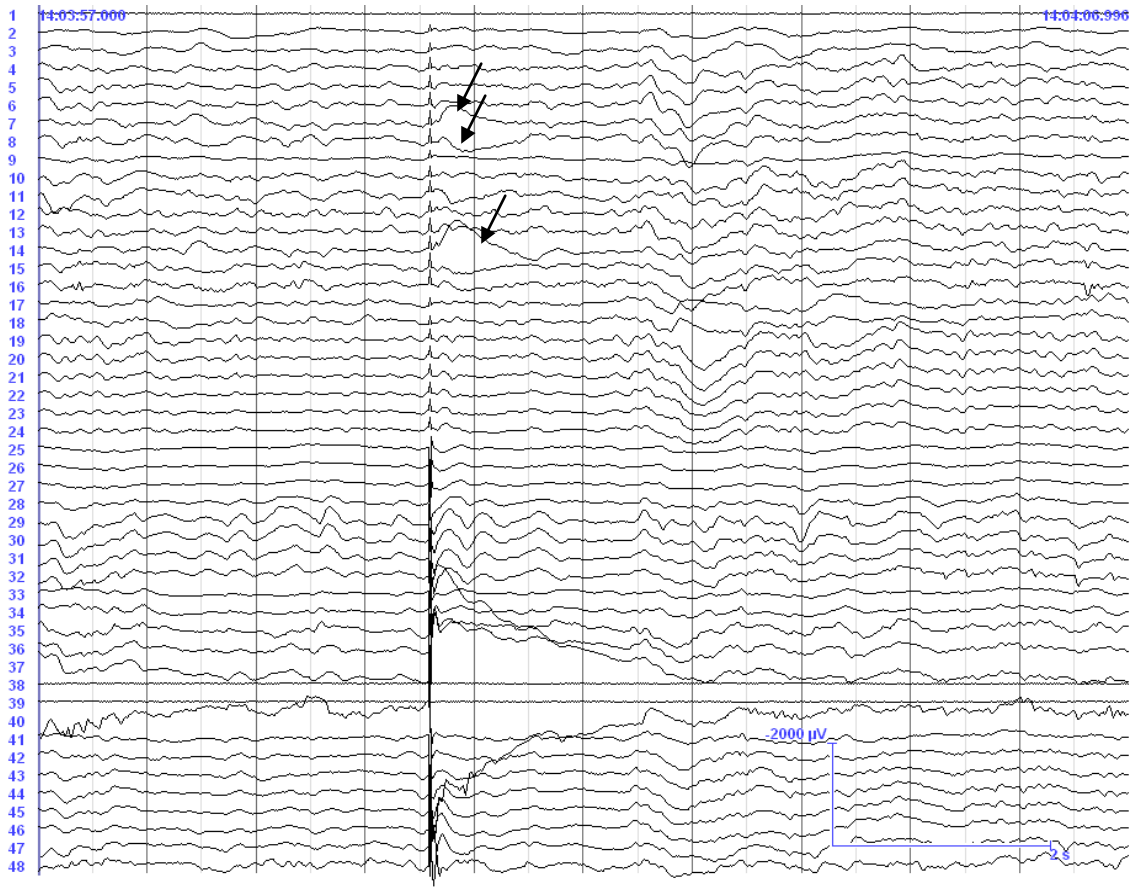


Figure 5. This and the following four figures illustrate the averaging process used to identify the response to the second PPES pulse. This figure shows an example of SPES response before averaging (arrows pointing to channels 7, 8 and 14). The vertical line corresponds to the stimulation artefact induced by SPES. Stimulation was delivered through the deepest contacts of left mid-temporal area (contacts 2 and 3) represented by flat lines in channels 39 and 38.

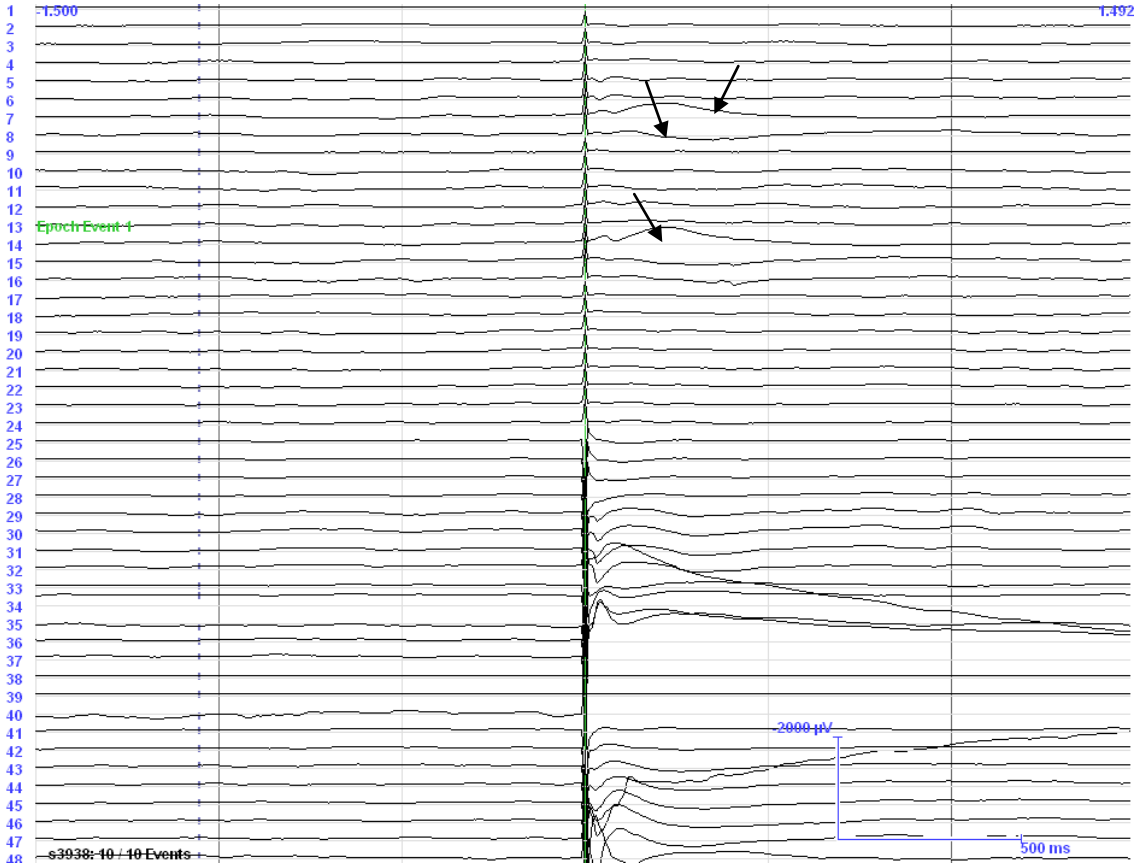


Figure 6. This figure shows an example of SPES response after averaging (arrows pointing to channels 7, 8 and 14). The vertical line corresponds to the stimulation artefact induced by SPES. Stimulation was delivered through the deepest contacts of left mid-temporal area (contacts 2 and 3) represented by flat lines in channels 39 and 38.

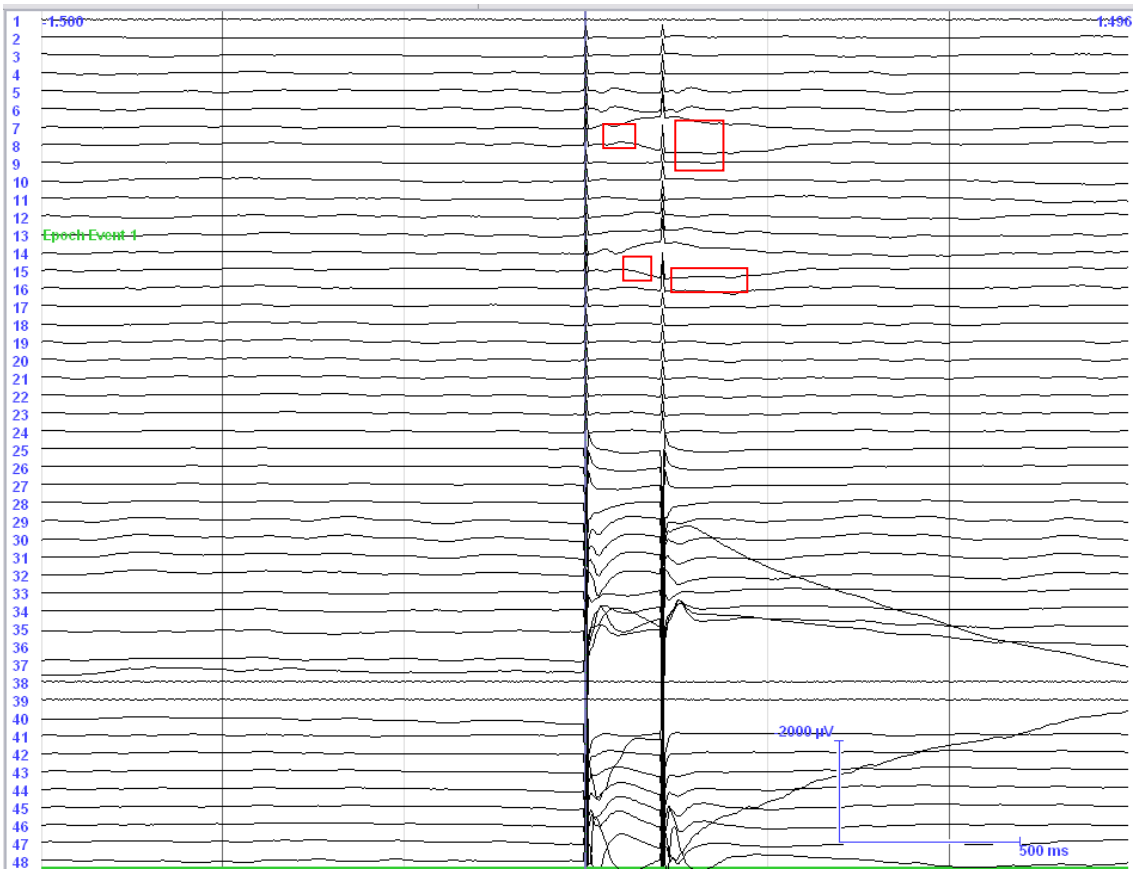


Figure 7. Example of 10 averaged paired pulse responses. The vertical lines 200 ms apart correspond to the stimulation artefact. The responses elicited by the first and second pulse of PPES are highlighted in red rectangles. Note that the response to the first pulse lasts for longer than the interstimulus interval (200 msec). Consequently, the response to the first pulse overlaps with the response to the second pulse. Stimulation was delivered through the deepest contacts of left mid-temporal area (contacts 2 and 3) represented by flat lines in channels 39 and 38.

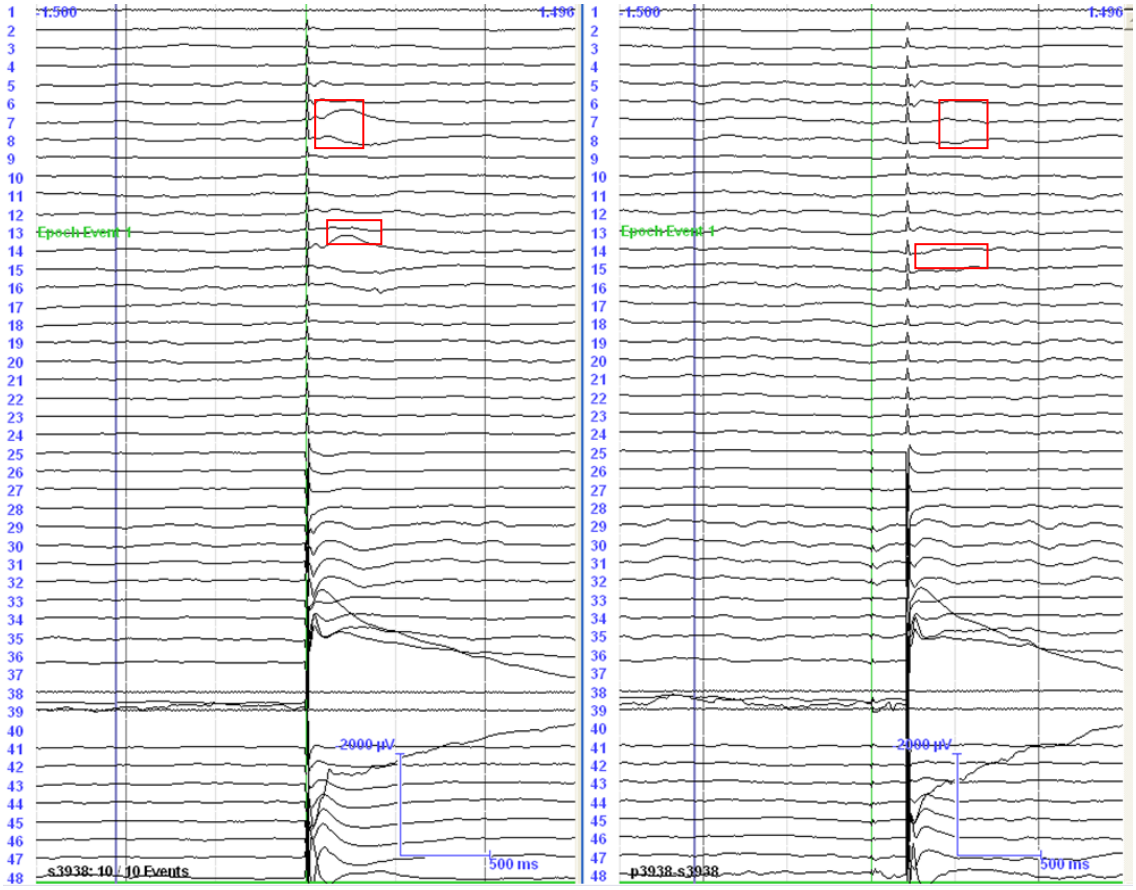


Figure 8. Example of suppression in channels 7, 8 and 14 (red rectangles). The averaged SPES response is seen on the left side of the figure. The right side of the figure shows the averaged PPES response after subtraction of the SPES response in order to remove the response to the first pulse from the response to the second pulse of PPES. For such subtraction, the SPES response has been superimposed on the green vertical line on the right hand panel, 200 ms before the second PPES pulse, exactly where the artefact to the first PPES pulse would have been (see figure 3). Consequently, stimulation artefact and response to the first PPES pulse are removed by this subtraction, and the result of this subtraction on the PPES recording (right hand panel) is the response exclusively elicited by the second pulse of PPES. Note the disappearance of the SPES response (left) on the PPES response (right), suggesting the presence of suppression. Stimulation occurs at the deepest contacts of left mid-temporal area (contacts 2 and 3) represented by flat lines in channels 39 and 38.

C. PROCESSING THE DATA

Even when a relatively long interpulse interval of 200 ms is used, a difficulty in analysing the response to the second pulse arises from the fact that the duration of the response to the first pulse often exceeded the interpulse interval. Consequently, the response to the second stimulus adds to the response from the first pulse. To overcome this difficulty**,** the response elicited by SPES was subtracted from the response elicited by the first stimulus of PPES. The result of this subtraction is the response elicited by the second pulse of PPES. This response to the second pulse was compared to the response elicited during SPES in order to establish if the response to the second pulse was depressed or facilitated.

Processing requires two stages:

a) **Averaging** of responses to identical stimuli corresponding to the same stimulation trial. Averaging was carried out automatically synchronising with the stimulation artefact identified with the amplitude threshold determined as described above.

b) Difference: for each stimulation intensity, polarity and topography, the averaged response to SPES is subtracted from the averaged response induced by the first pulse of the PPES. The result of this subtraction on the PPES recording is the response exclusively elicited by the second pulse of PPES (figure 8 above). The response to the second PPES pulse is compared to the response elicited during SPES in order to establish if the response to the second PPES pulse is suppressed, depressed or facilitated. The distribution of depression/facilitation/suppression is then compared with seizure onset area during the data analysis described below. Remaining stimulation artefact was removed by the ASA software which contains a facility for automatic correction of stimulation artefact built into the commercial software and based on the use of bandpass filters.

D. PRELIMILARY PILOT STUDIES

Since the study includes a large number of variables, four pilot studies were carried out to optimise the choice of variables for the main data analysis (section E). More specifically, pilot studies were carried out to:

- estimate the effects of interpulse interval on responses,
- estimate the effect on response amplitude of the jitter in the detection of stimulus artefact,
- estimate the effect of the artefact correction facility of the ASA system on the amplitude of responses,
- choose of the best response type to study.

D.1. Estimation of the effects of interpulse interval on responses

As reviewed in the introduction, the duration of the interpulse interval can affect the amplitude of responses to the test stimulus of PPES. In this study, the choice of 200 ms interpulse intervals was largely based on clinical grounds, as since 2000 this is the interval most commonly used in the presurgical assessment protocol at King's College Hospital, because this interval appears to be particularly effective in inducing delayed responses. Nevertheless, to investigate the effects of interpulse interval, a pilot study was undertaken in 7 patients who were studied with several interpulse intervals (3 patients studied with the Telefactor System and 4 patients studied with the Nervus System). Responses were recorded to stimulation to different interpulse intervals from 100 msec to 1000 ms in increments of 100 ms. Data were pre-processed and processed as described above. In each patient, the 3-4 channels showing the largest SPES responses were considered. The amplitude of the SPES response and the amplitude of the response elicited by the second PPES pulse were measured for all interpulse intervals and their difference and percentage ratio calculated (100 times the difference between response to first pulse, minus response to second pulse). Thus, a positive value for the percentage ration would indicate depression, a negative one facilitation while zero would indicate suppression.

D.2. Estimation of the effect on response amplitude of the jitter in the detection of stimulus artefact.

Since the duration of each stimulation pulse was 1 ms and the sampling rate was 200 or 256 Hz (sampling periods of 5 or 4 ms), it is likely that the stimulation artefact would be aliased and, consequently, the stimulation artefact would not be detected in phase for different pulses belonging to the same trial. This jitter cannot be longer than ±2.5 ms or ±2 ms, depending on the sampling rate. This is unlikely to significantly affect the amplitude of the responses studied, which last for several hundreds of ms. Nevertheless, this pilot study was designed to estimate the influence of such jitter on the results. Four patients were included. Data were acquired with the Nervus System with a 4 ms sampling period. Data were pre-processed with the ASA system as detailed above. Analysis was performed before recordings were averaged. In every patient, the 3-4 channels showing the largest SPES responses were considered. The amplitudes of the responses to each SPES single pulse were measured manually with screen cursors. This generated 10 measurements for channel for each recording. One-way analysis of variance (ANOVA) was performed and the residual variance calculated with SPSS version 15.

D.3. Estimation of the effect of the artefact correction facility of the ASA system on the amplitude of responses.

This pilot study was undertaken to estimate the influence of artefact correction of the ASA system on responses. The study included 4 patients studied with the recording obtained with the Nervus System. Apart from one, the 4 channels with the largest SPES response were considered in each patient. Data were pre-processed and processed as described above. The amplitudes of the SPES responses and responses to the second pulse of PPES were measured manually with screen cursors before and after artefact correction. The degree of depression or facilitation was calculated as a ratio (percentage) of the amplitude of the SPES response to the amplitude of the response elicited from the second PPES pulse of the paired pulse (SPES/PPES). Both ratios were calculated before and after artefact correction. Ratios before and after artefact correction were compare with the paired T-student test.

D.4. Choice of the best response type

This pilot study was designed to estimate which response type (facilitation, depression or suppression) could be more specific as a marker for the seizure onset area. This study included 7 patients displaying focal seizure onset. Data were acquired with the Nervus System. Data were pre-processed and processed with the ASA software as described above. Presence of facilitation, depression or suppression was assessed by visual inspection of the responses to SPES and PPES. For each patient, a table with all responses to SPES and PPES was created per patient. Each column represents an electrode and four different colours were used in order to facilitate visualisation of the distribution of facilitation, depression and suppression. Facilitation was represented by green boxes, depression by yellow boxes, suppression by red boxes and no-change (similar amplitude for SPES and second PPES responses) was represented by light blue boxes. Stimulating electrodes were represented by black and dark blue colour (see figure 9). Then, the percentage of facilitated, depressed and suppressed paired pulses in SO (seizure onset) area (or lobe) was measured.

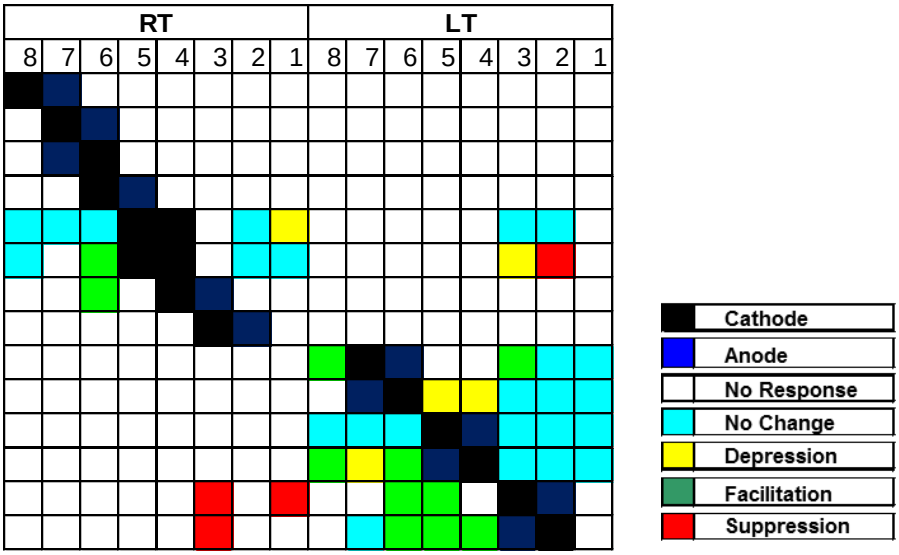


Figure 9. Example of a checkerboard diagram from a patient with bilateral subtemporal strips. The first row from the top shows the label of the implanted electrodes according to location (RT=right temporal and LT= left temporal lobe). The second row shows the electrodes (contacts) number (8 being most superficial and 1 being deepest). Rows below the second row show the distribution of response type associated with stimulation of each pair of contiguous electrodes (shown in black and dark blue). Each row represents results from a stimulation trial, and each column represents an electrode contact. For each row, the electrode used as cathode appears in black and the electrode used as anode appears in dark blue. For stimulation with each pair of electrodes, the associated responses are read horizontally, in the same row. Absence of response to the second pulse appears as white squares, facilitation as green, depression as yellow, suppression as red and no difference in response amplitude as light blue. For example, when contacts 5 and 4 (with 5 as cathode) of the right temporal lobe are used to stimulate, no change in amplitude was seen in the right temporal electrode contacts 2, 6, 7 and 8 and depression was recorded in contact 1. In addition, no change responses were seen in contacts 2 and 3 if the left temporal electrode.

E. MAIN DATA ANALYSIS

Data analysis aimed at establishing if there is a relation between the location of the different response types and the seizure onset zone or surgical outcome. This was achieved through the following 3 stages:

- Identification of the following ***relationships*** among the averaged response to SPES and PPES:
    - ***No change***: no difference in amplitude between the response to SPES and the response to the second pulse of PPES.
    - ***Suppression:*** absence of an identifiable response to the second pulse of PPES in the presence of a response to SPES.
    - ***Depression:*** reduced response to the second pulse of PPES compared to the amplitude of the response to SPES.
    - ***Facilitation:*** increased response to the second pulse of PPES compared to the amplitude of the response elicited by SPES.
- Confirming if there is an association between the seizure onset area and the topography of each condition.
- Determining if there is an association between surgical outcome and the removal of areas showing each condition.

E.1. Identification of suppression, depression and facilitation

The amplitude of the responses evoked by the second stimulus is compared with the amplitude of the responses evoked by SPES. In order to improve signal to noise ratio, this was carried out on averaged responses as stated above. For the identification of suppression, both visual and automatic methods were applied. As opposed to suppression, identification of depression and facilitation quite often required calculation of differences in the amplitude between the response elicited by the first pulse and the response elicited by the second pulse of the paired pulse. Such differences were often small, and therefore visual analysis was considered to be at least impractical and difficult. Consequently, for depression and facilitation, an automatic system of analysis was developed as described below.

E.1.1. Visual analysis

Visual analysis was applied for all 79 patients. Suppression was the only condition type investigated visually. Suppression was identified by the absence of a response above noise level to the second PPES pulse in the presence of response to SPES. An example of this method can be seen in figure 8**.**

E.1.2. Automatic analysis

This method was developed in order to objectively detect all condition types: suppression, depression, facilitation and no change. An automatic programme based on Matlab was initially developed to read Nervus files. Consequently automatic analysis was applied only to the recordings obtained with the Nervus system (55 patients). This initial programme was further developed and optimised by Dr Amir Eftekhar a postgraduate researcher at the department of electrical and electronic engineering of the Imperial College (figure 10-the final programme can be seen in the Appendix). As shown in table 2, the software analyses the data in three stages. First, the data is loaded in memory (imported). Second, responses are identified and measured. Third, the amplitudes of responses to SPES and to the second pulse of PPES are compared. The specific steps performed by the software are described in the following paragraphs.

First, the PPES and SPES data before and after artefact correction by the ASA system were imported. Once imported SPES and PPES response amplitude were measured and compared automatically as follows:

<u>*SPES analysis*</u>:

- *Stimulus detection* – In the averaged traces before artefact correction, the stimulus artefact is detected by the software in order to search for the response on which to measure amplitude. The stimulus artefact was identified by finding the maximal amplitude in the signal.

* *Defining response time window* – A time window of 0.5 seconds was chosen on the traces after artifact correction in order to search for SPES responses to stimulation (the response time window). The beginning of the response time window was defined by the location of the stimulus artifact. As the stimulation artifact lasts for longer than one EEG sample, a point within the stimulation artifact had to be defined as the starting point for the response time window. This was the point where the amplitude of the second flank of the SPES artefact had a value equal to the maximal amplitude of the artefact divided by 100. Contingency was made for the case when the artefact induced amplifier saturation and consequently the signal does not reach this amplitude. In these cases, a fixed point after the artefact was chosen based on trial and error, in order to define the beginning of the response time window.

* *Defining the Response* – The response is usually a biphasic, sinusoidal-like waveform. In some cases there is a second delayed response of similar characteristics. The responses can have different polarities: minima precede maxima or vice versa. Consequently, the software first extracts the largest maxima and largest minima in the response time window. If the minima precede the maxima, the software also searches for maxima in the period before the minima, and for minima in the period after the maxima. Hence, at most 2 minima and 2 maxima are identified. These responses are also evaluated based on their relative temporal position in the response time window and their amplitudes relative to the noise level. Interestingly, in many cases, a triphasic response was seen, some of which are shown in figure 11. Once extracted, two parameters were used for further analysis: a) the largest difference between the maxima and the minima (i.e. **the largest response**), and b) the difference between the maxima/minima pair closest to the stimulus artefact (i.e. **the earliest response**).

PPES analysis: The following two methods were used for the analysis of PPES responses.

i. *Same latency*: Here, we assume that the response elicited using PPES should be in the same or similar temporal position to the SPES response. The response time window for PPES is defined in the same way as detailed above for SPES. Maxima and minima are searched in the ±25 ms window before and after the maxima/minima of the corresponding SPES response.

ii. *Any latency*: This method extracted the response in exactly the same way as the SPES analysis, i.e. identify artefact and extract maxima/minima. This method was designed to compare responses where responses to SPES and to second pulse of PPES had different morphology (i.e. measurements at around the same latency did not correspond to equivalent deflections).

Comparing SPES and PPES: Firstly, the baseline noise level is estimated by calculating the mean and standard deviation of the EEG samples on the averaged EEG during the 1.5 seconds immediately preceding the SPES/PPES artefact. Noise Amplitude Level of SPES (NALS) is defined as five times the standard deviation of the baseline noise level.

$$NALS = (\pm)5 \cdot SD\ (S_1, S2, \ldots\ldots\ldots\ldots\ldots S_n)$$

Where: SD = standard deviation

$(S_1, S2, \ldots\ldots\ldots\ldots\ldots S_n)$= EEG samples obtained during the baseline period, before stimulation.

Once the amplitude of the averaged responses is measured by the automatic software, the amplitude of the response to SPES is subtracted from the amplitude of the response to the second pulse of PPES. This difference is compared with the baseline noise level. The following four conditions are then identified according to the following criteria:

a) *No SPES response*: when the amplitude of the measured response to SPES is below NALS.

b) *No change*: If there is SPES response and the absolute value of the difference between the amplitudes of the response to SPES and the response to the second pulse to PPES is below NALS.

c) *Suppression*: If there is SPES response but the amplitude of the response to the second pulse of PPES is below NALS.

d) *Facilitation*: If all three following conditions are met:

i. There is SPES response,
ii. The absolute value of the difference between the SPES response and the response to the second pulse of PPES amplitudes is above NALS, and
iii. The amplitude of the response to the second pulse of PPES is higher than the amplitude of the response to SPES.

e) *Depression*: If all three following conditions are met:
i. There is SPES response,
ii. The absolute value of the difference between the SPES response and the response to the second pulse of PPES amplitudes is above NALS, and
iii. The amplitude of the response to the second pulse of PPES is lower than the amplitude of the response to SPES.

In summary, four combinations of automatic methods are possible for comparison of SPES and PPES responses:

a) same latency and largest response;
b) same latency and earliest response;
c) any latency and largest response;
d) any latency and earliest response.

E.2. Association between condition type and seizure onset (SO)

Seizure onset area(s) and lobe were identified in the ictal records of each patient. Once the presence of no change, suppression, facilitation and depression has been identified, the next step is to establish if each condition tends to occur in the cortical areas that generate epileptic seizures (i.e. the seizure onset zone). This task is complicated due to the large number of recording electrodes and stimulated sites. To simplify visualisation of the relation between condition type and seizure onset, a checkerboard graph was designed for each patient. A bi-dimensional checkerboard was created containing one column for each recording electrode and one row for the results of each stimulation trial. For each

stimulation trial, the pair of stimulating electrodes is represented by black and dark blue pixels (black was the cathode and dark blue the anode). For each stimulation trial, the distribution of each condition seen is shown by coloured pixels in the corresponding row: red for suppression, yellow for depression, green for facilitation and light blue for no-change. Checkerboard graphs were constructed for visual and automatic analyses, according to the following guidelines:

*E.2.1. Visual analysis***:** Depression and facilitation are quantitative measures where computer was essential in assessing their presence and magnitude (see below). Consequently, suppression was the only condition investigated with visual analysis, as suppression is essentially a qualitative condition (i.e. it is present or absent). During the creation of the checkerboard graphs, I was blind to seizure onset zone and clinical information from the patients studied.

*E.2.2. Automatic analysis***:** To visualise the regions showing suppression, depression or facilitation, a computer routine in the software developed automatically displayed the checkerboard graphs described above. The graphs were displayed corresponding to a NALS threshold of 10 SD (±5 SD) as baseline levels (an example is shown in figure 12).

The presence of each condition was compared to the seizure onset lobe and for suppression to seizure area(s). ***Seizure onset lobe*** was the lobe where seizure onset was observed on intracranial recordings. ***Seizure onset area*** was the area underlying the electrodes showing seizure onset. After such a comparison conditions were classified as occurring: a) exclusively in SO, b) in SO and in other areas (SO+), or c) exclusively outside SO.

E.3. Association between surgical outcome and removal of areas showing each condition type:

Neuroimaging and medical notes were reviewed to establish if areas showing suppression had been removed.

Only patients with a follow up period longer than 9 months were included. Among the 79 patients, 38 were operated and 26 had a follow up period longer than 9 months.

E.3.1. Neuroimaging

Neuroimaging was not used for analysis in this thesis. However, it was one of the key methods used to decide the implantation sites during clinical presurgical assessment. Brain MRI scans before and after resection were performed in all patients. The MRI protocol followed at King's College Hospital included the following parameters:

a) Coronal fast spin echo T2-weighted (TEeff 85 ms TR 4300 ms) 3.5mm slice thickness, 0.5mm gap, perpendicular to temporal horn.

b) Coronal FLAIR (fluid attenuated inversion recovery) (TE 115eff ms TR 8500 ms TI 1900 ms) 3.5mm slice thickness, 0.5mm gap, perpendicular to temporal horn.

c) Coronal IR-prepped SPGR T1-weighted (IR=inversion recovery, SPGR = spoiled gradient recalled) flip angle 30 TE 2.8 ms TR 14 ms 1.5mm partition.

d) Axial fast spin echo T2-weighted (TE 75eff ms TR 3500 ms) 5mm slice thickness 2mm gap, parallel to AC-PC line.

Unclear non-specific changes were characterised as non-lesional or *"normal"*.

E.3.2. Epilepsy Surgery

Thirty-eight patients underwent epilepsy surgery. Among the 27 patients with a temporal resection, 15 had left and 12 had right resections. Frontal resections were performed in 5 patients, 4 on the left and one on the right side. A right occipital cortical resection was carried out in another two patients. In one of the two cases with parietal resections, a right multiple subpial transection (MST) was also performed. One patient had thermocoagulation of a hypothalamic hamartoma. Finally, one patient had a right lateral occipital resection in addition to hippocampectomy.

The areas showing suppression were classified as: a) totally resected if the entire suppressed area was resected, b) partially resected if part of this area was resected and c) non-resected if the suppressed area was not resected at all.

Surgical outcome

Surgical outcome was classified in four grades according to Engel's classification (Engel, Jr. *et al.* 1993) :

Table 5.18.1. – Engel Outcome groups 1993		
Outcome		Definition
Group	subgroup	
I Free of disabling seizures[a]	a	Completely seizure free since surgery
	b	Non-disabling simple partial seizures only since surgery
	c	Some disabling seizures since surgery but free of disabling seizures for two years
	d	Generalized convulsion with anti-epileptic drug withdrawal only
II Rare disabling seizures	a	Initially free of disabling seizures but rare disabling seizures now
	b	Rare disabling seizures since surgery
	c	More than rare disabling seizures after surgery but rare disabling seizures for at least two years
	d	Nocturnal seizures only
III Worthwhile improvement	a	Worthwhile seizure reduction
	b	Prolonged seizure-free intervals amounting to greater than half the follow-up period but not less than two years.
IV No worthwhile improvement	a	Significant seizure reduction
	b	No appreciable change
	c	Worse
a – excludes early post-operative seizures, ie first few weeks.		

Table 1. Engel's classification for surgical outcome.

For this present study, patients with grades I and II were considered as having a *'favourable'* surgical outcome, whereas grades III and IV were considered as having a *'poor'* outcome.

DATA LOADING

1. Importing the data of PPES and SPES before and after artefact correction.

DATA ANALYSIS

1. Description of the area under investigation for SPES responses.
2. Calculation of the maximum and minimum amplitudes of the SPES responses and their latencies for both methods.
3. Description of the area to look for a PPES response according to the old method.
4. Calculation of the maximum and minimum amplitudes of the PPES responses in the already determined area with the old always method.
5. Description of the area to look for a PPES response according to the new method.
2. Calculation of the maxima and minima of the PPES responses according to the new method.

DATA COMPARISON

1. Definitions of suppression, depression, facilitation, similar and no response.
2. Comparison of the amplitudes of the SPES and PPES responses in both methods.
3. Plotting the data. Figures 1-3.

Table 2. Summarised stages of analysis carried out by the software developed in Matlab

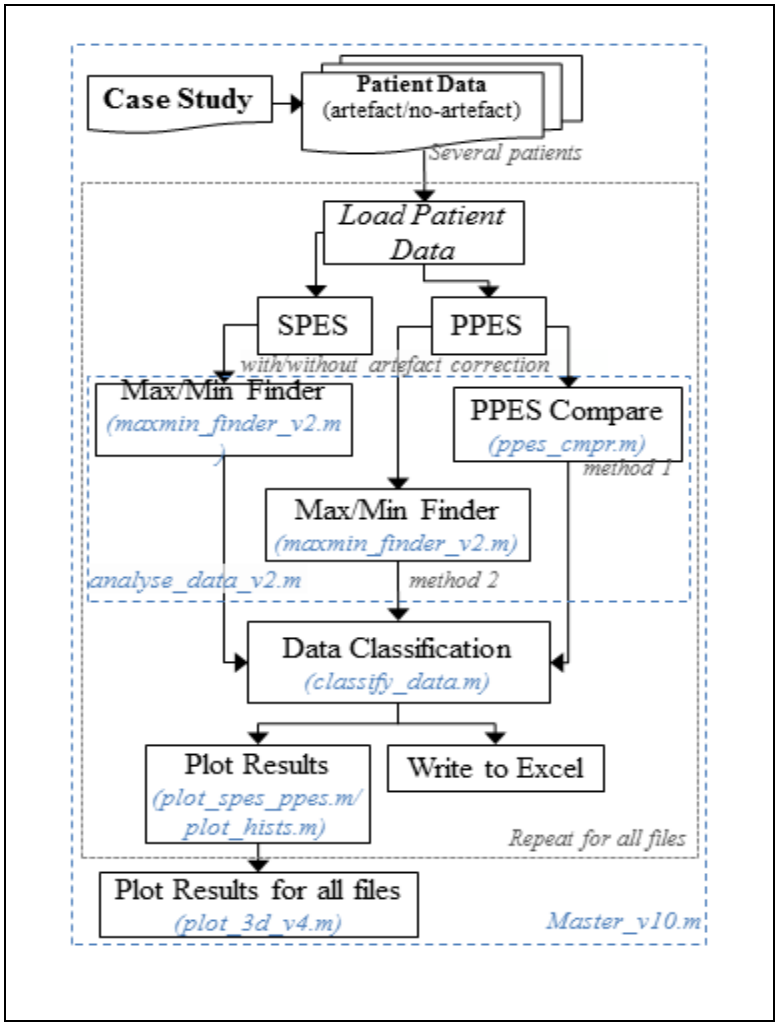


Figure 10. Flow-chart of the Matlab programme. The main computer function (Master_v10.m) contains the following steps: 1) data about the patient are loaded; 2) PPES and SPES data are loaded before and after artefact correction; 3) the function maxmin_finder_v2.m calculates the maximum and minimum amplitude of the SPES responses; 4) the window frame for method 1 is identified to look for PPES responses (function ppes_cmpr.m); 5) the maximum and minimum amplitude of these responses are calculated using the same function as before. The 4th and 5th steps are repeated for method 2 for PPES responses; 6) data are classified (classify_data.m function) as suppression, depression, or facilitation; 7) plotting and saving the data is performed before repeating the steps for another patient.

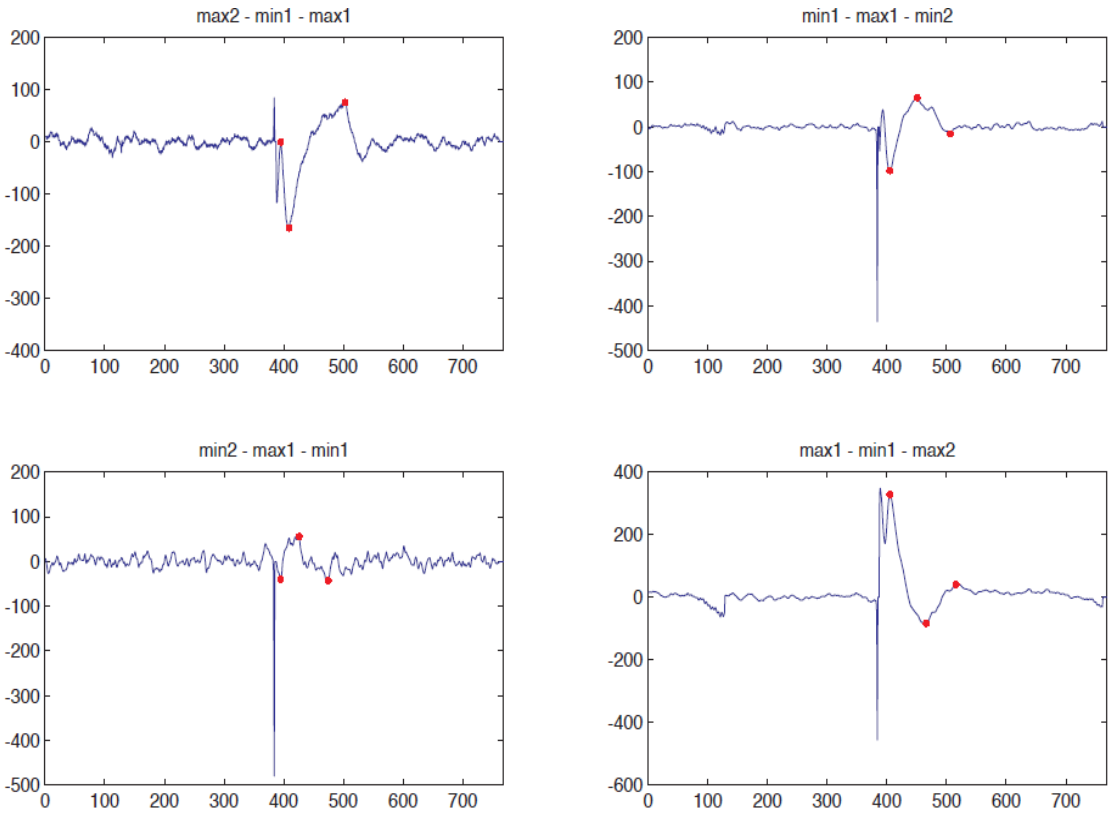


Figure 11. SPES responses with different morphologies are shown. The red dots correspond to the maxima and minima of each trace. In the top traces min1 precedes max1 whereas in the bottom traces min1 follows max1.

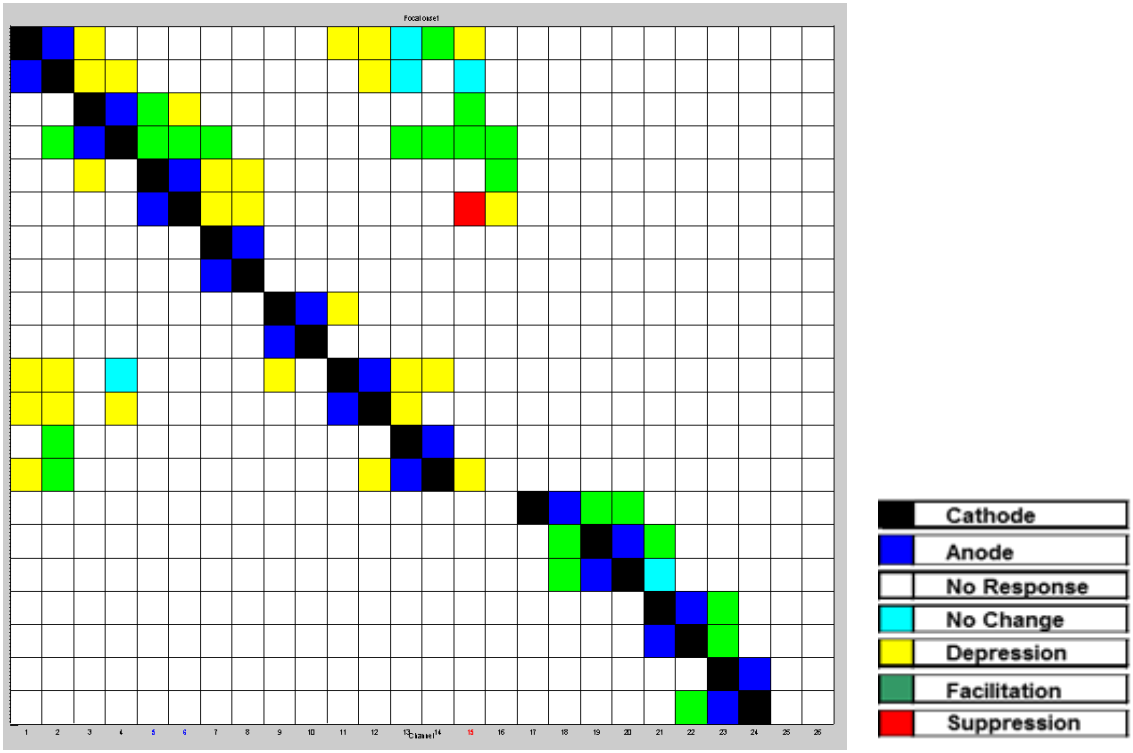


Figure 12. This example includes all conditions at 10 std. Figures 18-20 belong to the same patient. Each column represents a recording channel (channel 1 to the left). Each column represents responses (conditions) seen during a stimulation trial. Location of responses to stimulation is shown as coloured squares (see legend on the right of the figure) corresponding to the channels where responses are seen on the same row. For instance, the top row indicates that when stimulating with electrode in channel 1 as negative and electrode in channel 2 as positive, depression was recorded in channels 3, 11, 12 and 15, facilitation was seen in channel 14, and no change in the responses to first and second pulses was seen in channel 13.

E.4. Statistical analysis

Statistical analysis was performed to show the relation between the resection of the area showing suppression and the surgery outcome. The Fischer's exact test was used for such analysis. The test was performed only in temporal patients. Due to the limited number of the patients included in the other groups a simple description of this relation was carried out. The following link was used:
http://www.graphpad.com/quickcalcs/contingency2.cfm
Proportions were compared by using the Z statistic, two tailed at a significance level of 0.05. The calculator used can be found at:
http://www.philosophyexperiments.com/statistics/

SPSS v 15.0 was used for jitter analysis,

RESULTS

RESULTS

1. SUBJECTS AND SEIZURES

The study includes 79 patients (40 males and 39 females, mean age 38 years, range of 15 to 66 years). During the telemetry period, an average of seven seizures (range 0 to 45) was recorded per patient.

2. MRI FINDINGS

Neuroimaging was performed in all 79 patients. Abnormal MRI findings were seen in 45 patients, and normal or non-specific MRIs were seen in 34 patients.

Thirty-two patients had MRI temporal abnormalities. Twenty-six patients showed medial temporal sclerosis (MTS) (17 left, eight right, and one bilateral), one patient showed heterotopia in the right posterior temporal cortex, three patients showed temporal focal cortical dysplasia (FCD), and two patients showed dysembryoplastic neuroepithelial tumour (DNET) .

Four patients had frontal MRI abnormalities. Three patients showed focal cortical dysplasia and one patients showed and infarct.

Nine patients had MRI abnormalities outside the temporal or frontal lobes. One patient showed multifocal ischaemic lesions, one an hypothalamic hamartoma, one a DNET in the right occipital region, one had FCD over the right inferior parietal area, one had heterotopia in the left occipito-temporal region, one subependymal heterotopia, one a dysplastic lesion or a ganglioganglioma over the left parietal lobe, one an extensive cyst over the left hemisphere, and one patient had Parry-Romberg syndrome.

3. ELECTRODE PLACEMENT

Localisation and lateralisation of recording electrodes ***(Table 3)***

Table 3. Distribution of electrode types in the 79 patients studied. T=Temporal; ET=extra-temporal

TYPE OF ELECTRODES	BILATERAL	UNILATERAL	N
Subdural T	22	2	24
Subdural ET	1	1	2
Subdural T+ET	13	20	33
Depth T	6	1	7
Depth ET	4	0	4
Depth T+ET	5	2	7
Subdural and Depth T	0	1	1
Subdural and Depth T+ET	0	1	1
Total	**51**	**28**	**79**

Subdural mats and strip electrodes were implanted in 59 patients. Among these, 24 had electrodes implanted exclusively in the temporal lobe, two had electrodes implanted exclusively in other lobes (frontal, parietal and occipital), and 33 had subdural electrodes in temporal and extra-temporal regions (table 3).

Intracerebral (depth) electrodes were implanted in 18 patients, among which seven patients had electrodes implanted exclusively in the temporal lobe, four patients exclusively in extra-temporal areas, and seven patients had depth electrodes implanted in temporal and extra-temporal regions.

A combination of subdural and depth electrodes was used in two patients, one with the electrodes located over the temporal lobes, and one with temporal and occipital electrodes.

In the 79 patients, the average percentage of contacts in seizure onset lobe for all patients with an identifiable seizure onset was 64%. In focal seizure onset patients, 58% of the total number of contacts was located in seizure onset lobe as opposed to 68% for regional

seizure onset patients, 56% for the independent focal and regional seizure onset patients and 86% for the bilateral seizure onset patients.

In the 55 patients where both methods of analysis (manual and automatic) were performed, the average percentage of contacts in seizure onset lobe was found to be 65%. In focal seizure onset patients, 58% of the total number of contacts was located in seizure onset lobe as opposed to 72% for regional seizure onset patients, 54% for the independent focal and regional seizure onset patients and 80% for the bilateral seizure onset patients.

4. TOPOGRAPHY OF SEIZURE ONSET (SO)

Among the 79 patients, 70 showed localised seizure onset (SO), three had the seizure onset area identified by functional stimulation. In six patients, telemetry was inconclusive, either because they did not have seizures during the telemetry period (two patients), or because they had widespread seizure onset involving both hemispheres (four patients).

Forty-nine patients were considered as having temporal epilepsy SO (26 left, 17 right, and six bitemporal).

Among the 10 patients with frontal SO, six had left SO, two had right SO, and two had bi-frontal SO.

Six patients had seizures originated independently from two lobes, four patients from the temporal and frontal lobes and two from temporal and occipital lobes.

Among the eight patients considered as having bi-lobar SO (SO involving two lobes), three had SO arising from the temporo-parietal region, one from the temporo-occipital region, two from the fronto-temporal region, and two from the fronto-parietal region. Two out of the eight patients had a left hemisphere onset in the temporo-parietal region, and six had a right hemisphere onset.

5. RELATION BETWEEN SEIZURE ONSET AREA AND SO TYPE (table 4)

Among the 49 temporal patients, 30 had focal seizures, 18 had regional seizures, and one had independent focal and regional seizures.

Five frontal patients had regional seizures, three had focal seizures and two patients had a bilateral seizure onset.

Among the six patients with seizures originated in independent lobes, three had regional seizures, one had focal seizures, one had bilateral seizures, and one had independent focal and regional seizures.

All eight patients with bi-lobar seizure onset, showed a regional seizure onset.

Table 4. Relation between seizure onset area and seizure type. The 6 patients with inconclusive seizure onset were not included. SO= seizure onset, F=Focal, R=regional.

SO area	Focal	Regional	F/R	Bilateral	Total pts
Frontal	3	5	0	2	**10**
Temporal	30	18	1	0	**49**
Independent SO involving two lobes	1	3	1	1	**6**
Widespread SO involving two lobes	0	8	0	0	**8**
Total pts	**34**	**32**	**2**	**3**	**73**

6. RESPONSES TO PAIRED PULSE ELECTRICAL STIMULATION

A total of 3,326 electrode contacts were implanted and stimulation was carried out through 1,663 electrode pairs. Responses to paired pulse electrical stimulation usually consisted of spikes, waves or spike-and-wave complexes. The morphology of the responses to SPES and to PPES tended to be similar, as they had identical stimulation parameters. An example of suppression, depression and facilitation can be seen in the following figures (figures 13, 14 and 15).

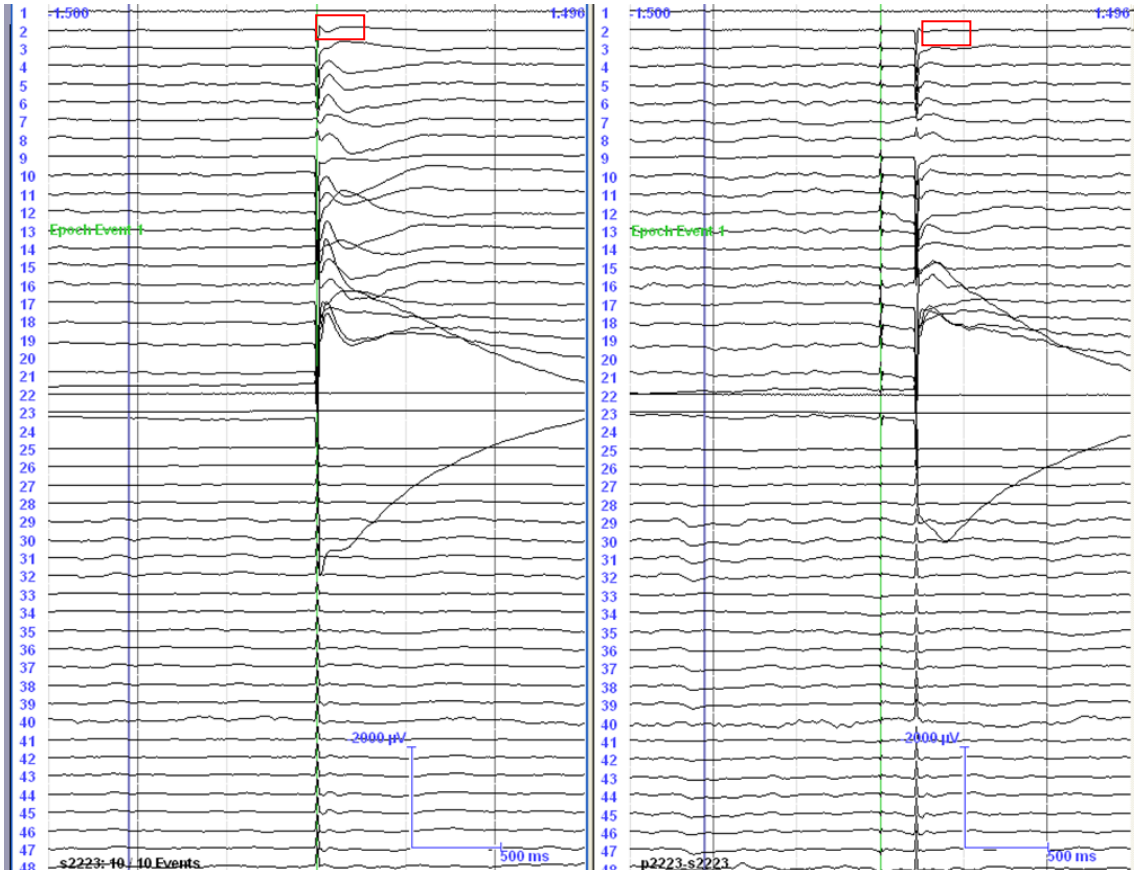


Figure 13. Example of suppression with the averaged SPES responses seen on the left and the averaged PPES responses seen on the right side of the figure. The SPES response and the suppressed response are highlighted by the red rectangle. Stimulation was delivered through the deepest contacts of right parietal area (contacts 3 and 2) represented by flat lines in channels 22 and 23.

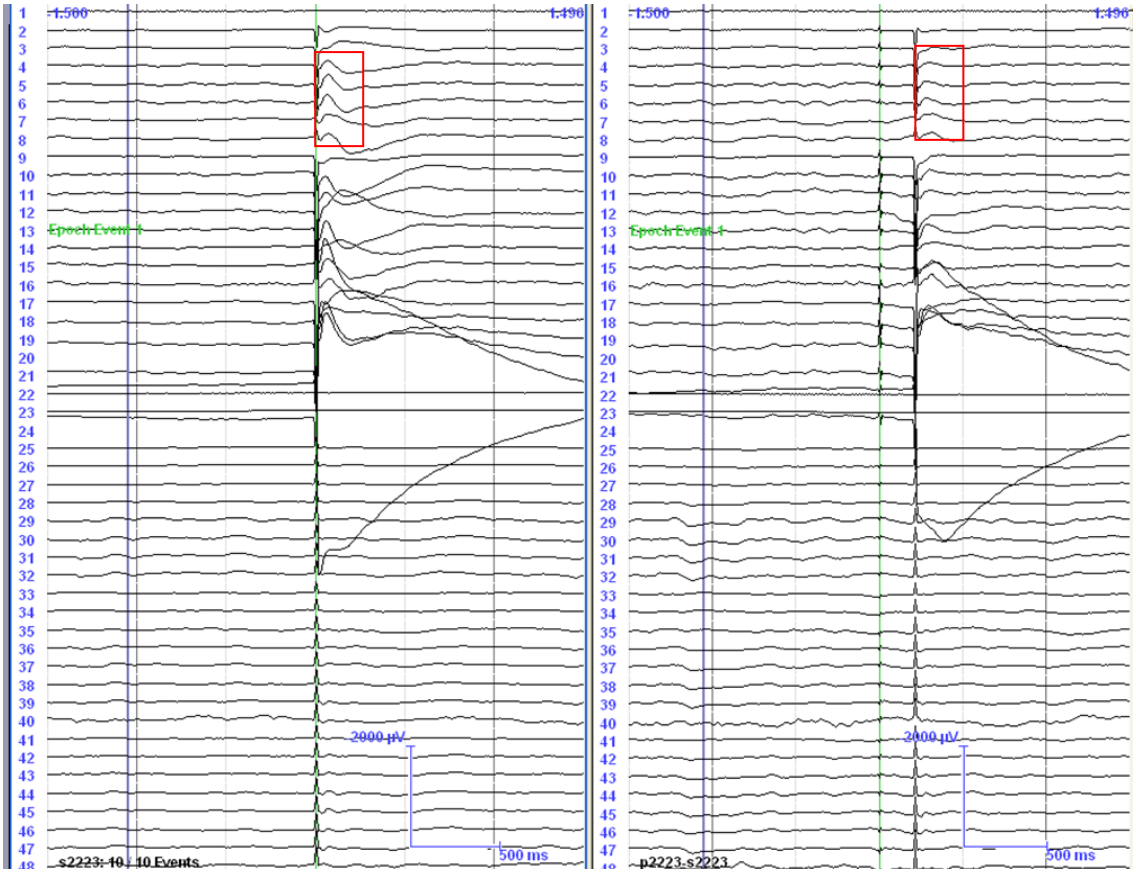


Figure 14. Example of depression. The averaged SPES responses are seen on the left side and the averaged PPES responses are seen on the right side of the figure. The SPES response and the response elicited by the second pulse of PPES are highlighted in red rectangles. Note the depression of the responses elicited by the second pulse of PPES (channels 4-8 or right anterior temporal contacts 5-1). Stimulation was delivered through the deepest contacts of right posterior temporal area (contacts 3 and 2) represented by flat lines in channels 22 and 23.

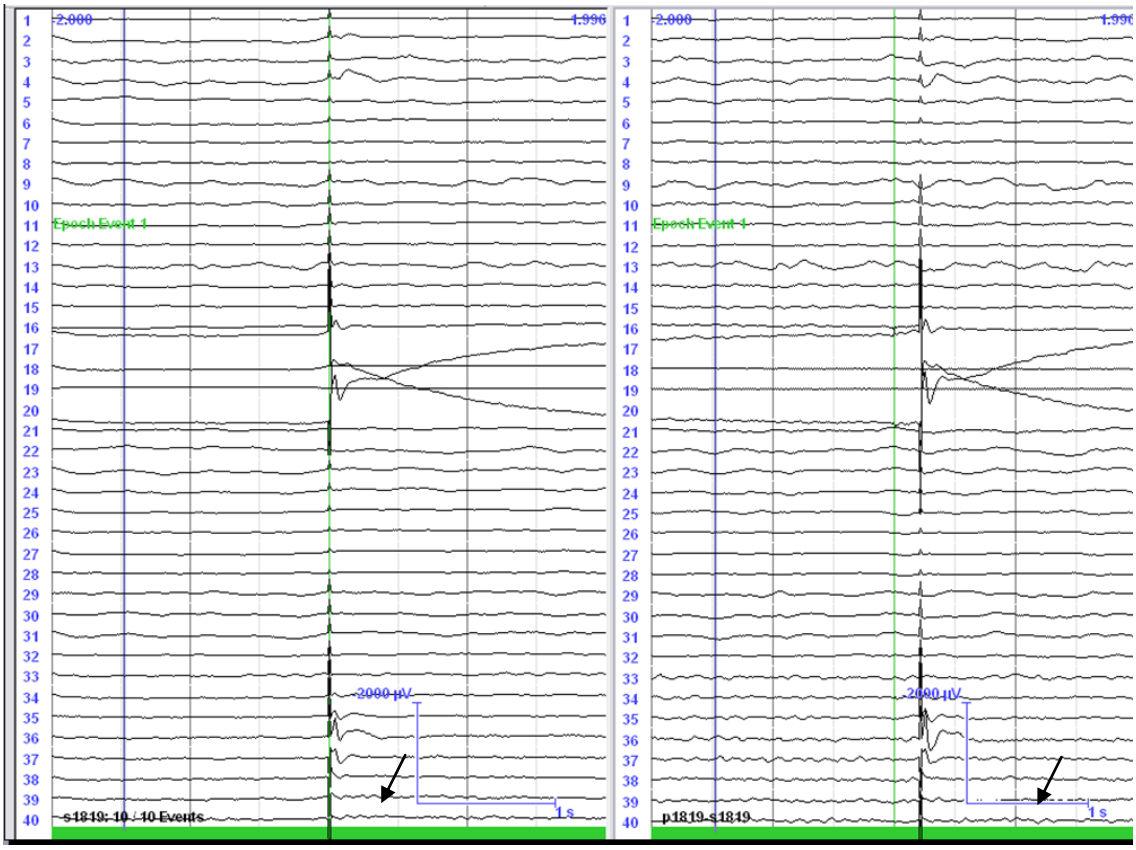


Figure 15. Example of facilitation. The averaged SPES response is seen on the left side and the averaged PPES response is seen on the right side of the figure. The SPES response and the response elicited by the second pulse of PPES are shown in black arrows. Note how the response elicited by the second pulse of PPES is greater compared with the SPES response (arrow). Stimulation was delivered through the deepest contacts of right parietal area (contacts 3 and 2) represented by flat lines in channels 18 and 19.

Four different types of conditions were seen when comparing the response elicited by the first pulse with the response elicited by the second pulse:

a) ***No change*** when the amplitude of the response to the second pulse is similar to the amplitude of the response to the first pulse.

b) ***Facilitation*** when the amplitude of the response to the second pulse is increased compared to the amplitude of the response to the first pulse.

c) ***Depression*** when the amplitude of the response to the second pulse is reduced compared to the amplitude of the response to the first pulse.

d) ***Suppression*** when the amplitude of the response to the second pulse is absent

7. RESULTS FROM PILOT STUDIES

7.1 Estimation of the effects of interpulse interval on responses

Figure 16 shows the average and maximal values of depression (bars above 0) and facilitation (bars above 0), and the summation of their average and maximal values for different interstimulus intervals in seven patients. Two-hundred millisecond intervals appear to induce the strongest facilitation and highest compound values for facilitation plus depression. Consequently, this interval was used for the remaining of measurements.

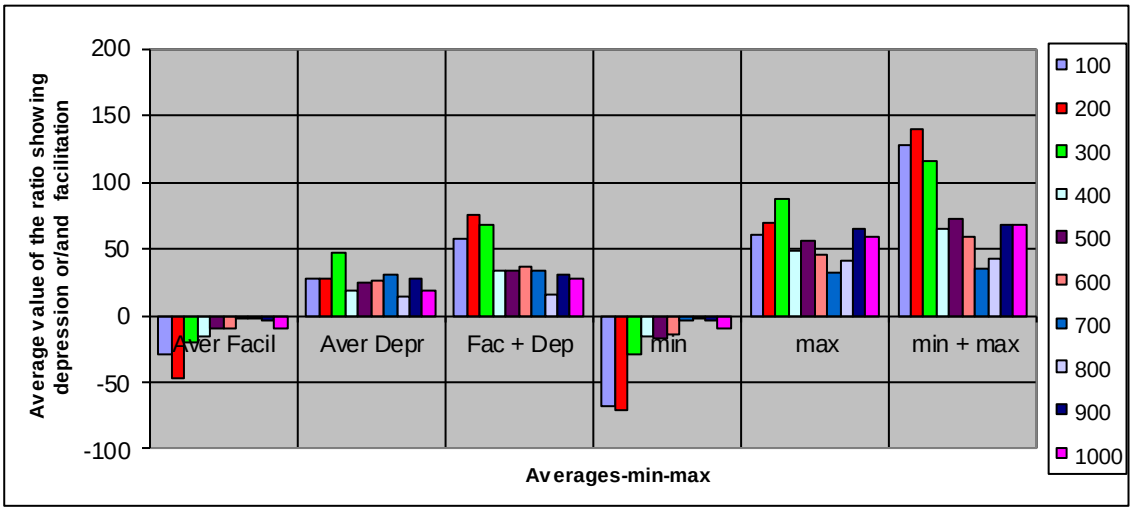


Figure 16. The average value of the ratio (see methods of pilot studies) indicating facilitation or/and depression is shown. Note (Fac+Dep, or min+max) that the IPI with the broadest spectrum of responses is the 200 msec. Aver=Average, Facil (or Fac) =Facilitation, Dep (or Depr) =Depression, min=minimum, max=maximum. Each colour represents a different IPI (look legend aside).

7.2. Estimation of the effect on response amplitude of the jitter in the detection of stimulus artefact.

Table 5 shows the results the average and standard deviations for the amplitudes (μV) of responses to SPES measured for each of the 10 identical stimulations belonging to the same trial. This was measured at 3-4 recording channels in each of the four patients included in this pilot study. ANOVA analysis showed a residual variance (within groups' variance) of 14.329, resulting in a standard deviation for measurements of 10 identical stimulations of 3.8 μV. This amounts to only 4.8% of the average signal amplitude (79 μV).

Table 5. Standard deviation (SD) for 10 response amplitude measurements induced by identical SPES stimuli. The residual variance is 14.329.

Patients (stimulation/channel)	**Average**	**SD**
Patient 1 LT9-10/LmT6	106.28	4.794163118
Patient 1 LT23-24/LT31	76.96	1.90682983
Patient 1 LT23-24/LT32	74.98	27.29460457
Patient 1 LT24-23/LT31	76.15	24.87641493
Patient 1 LT24-23/LT32	68.545	18.63896304
Patient 2 LT7-6/LT4	51.03	20.7379866
Patient 2 LT6-7/LT4	56.065	37.41802035
Patient 2 LT5-4/LT1	102.965	36.69059366
Patient 2 LT4-5/LT1	99.765	27.3569872
Patient 3 LpT5-4/LlatOcc2	73.38	27.00689345
Patient 3 LpT4-5/LlatOcc2	72.69	20.55647705
Patient 3LinfOcc2-1/LpT2	56.69	21.86712526
Patient 3LinfOcc1-2/LpT2	61.51	34.52886801
Patient 4 LmT5-4/LmT8	94.02	34.01709033
Patient 4 LmT4-5/LmT8	93.76	34.37489291
Patient 4 LmT7-6/LaT7	99.3	5.93932282

Patient 1 = left frontal seizure onset, Patient 2= bi-temporal seizure onset, Patient 3= Seizure onset independently from left temporal and left occipital lobes and patient 4= Seizure onset independently from left frontal and left temporal lobes. LT=left temporal, LaT=left anterior temporal, LmT=left mid-temporal, LlatOcc = left lateral occipital, LinfOcc =left inferior occipital.

7.3. Estimation of the effect of the artefact correction facility of the ASA system on the amplitude of responses.

The mean and standard deviations of the amplitude of SPES responses were calculated in four channels in each of the four patients included in this pilot study. Before artefact correction, the mean response amplitude was 109.54 µV and the standard deviation 57.04. After artefact correction, the mean response amplitude was 101.79 µV and the standard deviation 43. The two-tailed paired student t-test did not show significant differences before and after artefact rejection (15 degrees of freedom, t=1.868, p= 0.081).

7.4. Choice of the best response type

Figures 17 to 23 show checkerboard graphs displaying the distribution of suppression, depression, facilitation and no change estimated visually in the seven patients included in this pilot study.

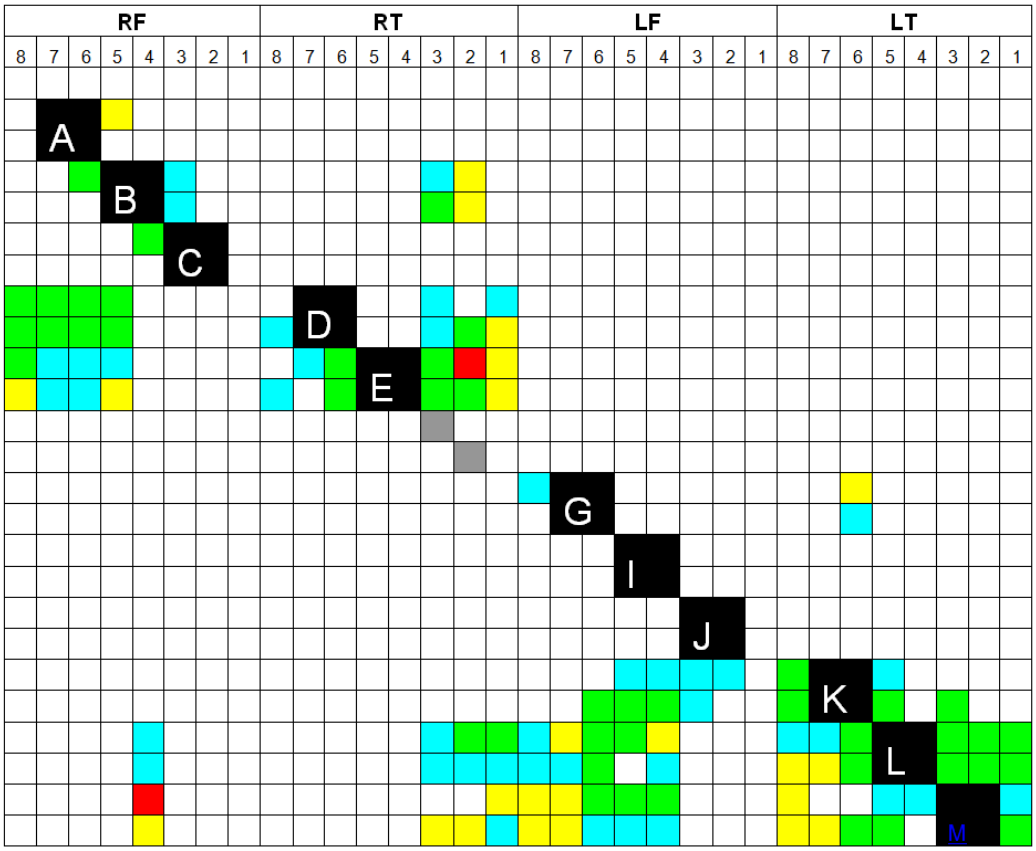


Figure 17. Patient with seizure onset on the left temporal lobe (LlatT3).

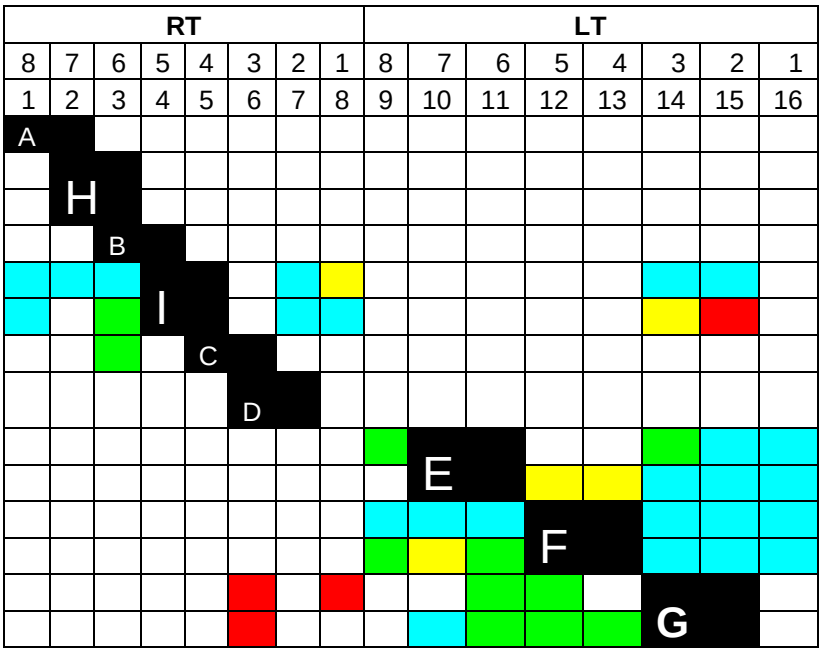


Figure 18. Patient with seizure onset on the left temporal lobe (contacts 1,2,3).

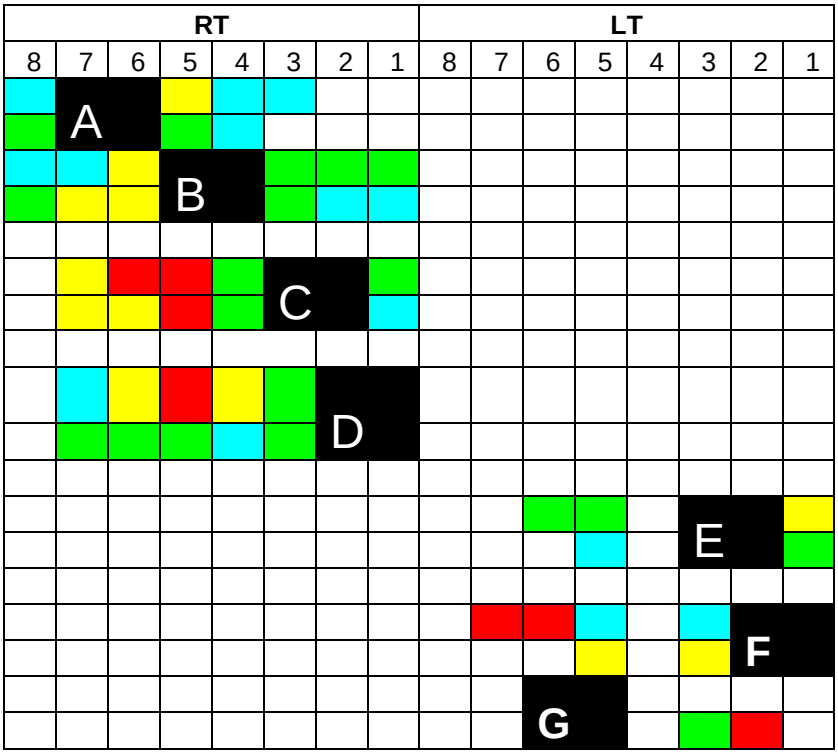


Figure 19. Patient with seizure onset on the left temporal lobe (contacts 5 and 6).

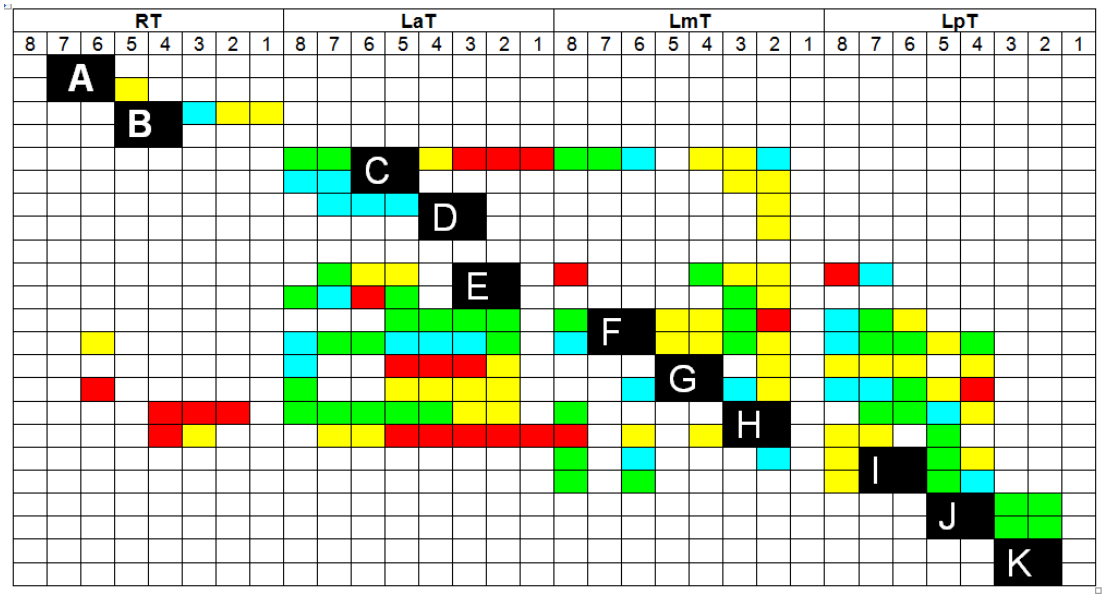


Figure 20. Patient with seizure onset is on the left anterior temporal lobe (contact 1).

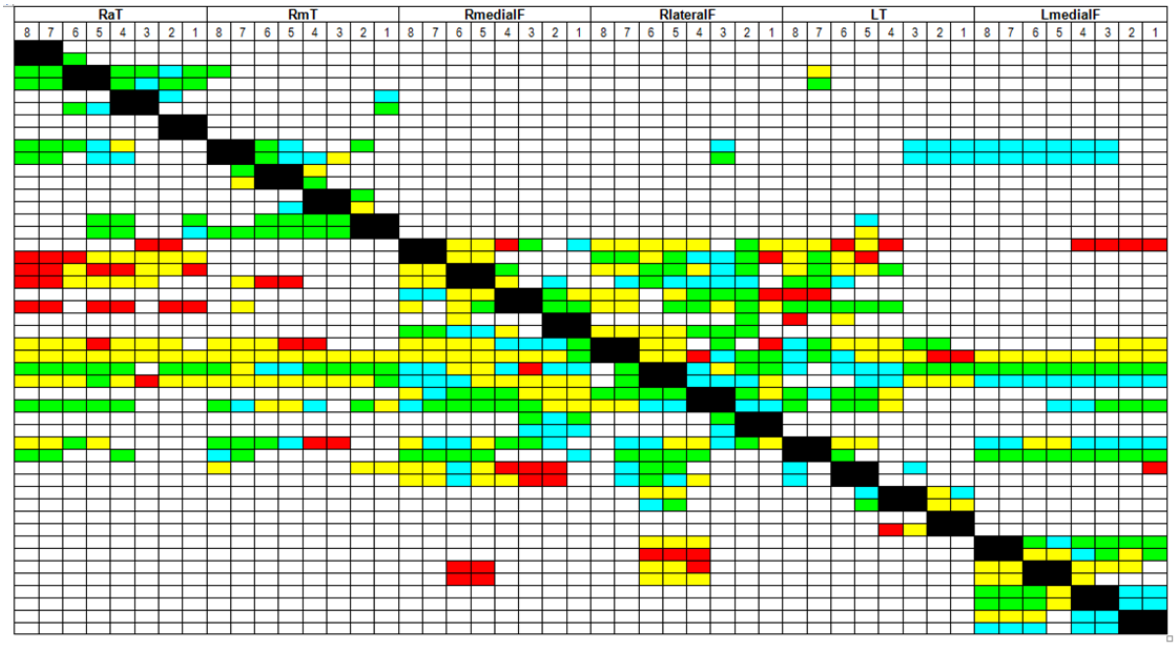


Figure 21: Patient with seizure onset is on the left temporal lobe (contact 7).

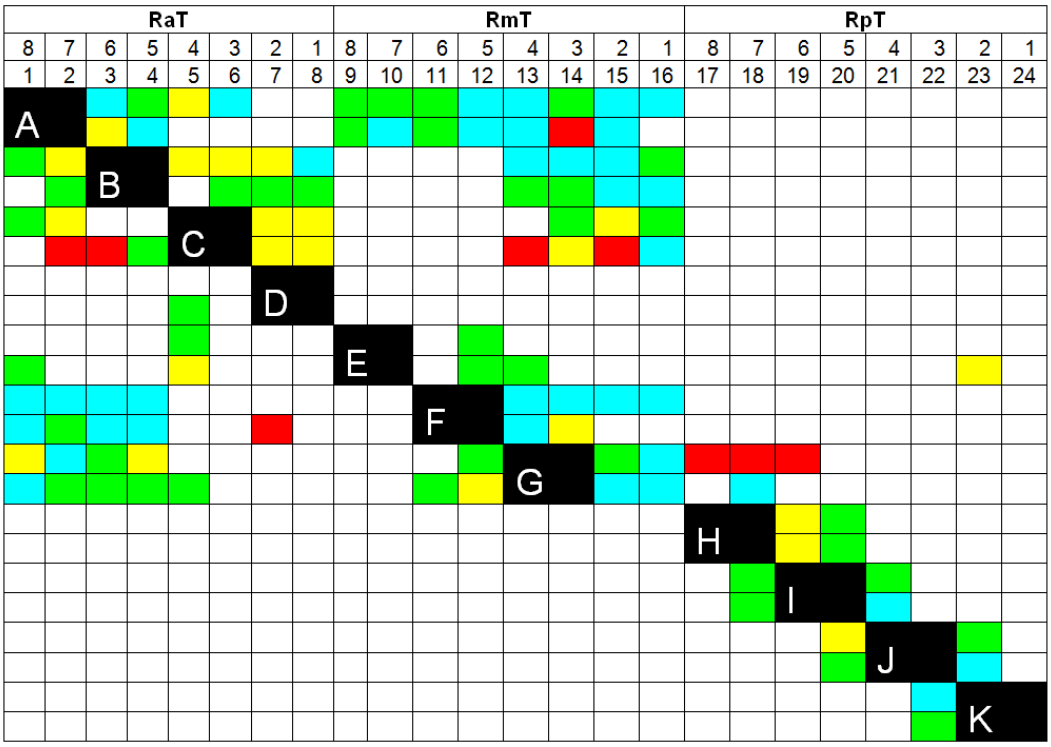


Figure 22: Patient with seizure onset is on the right anterior temporal lobe (contacts 3-4).

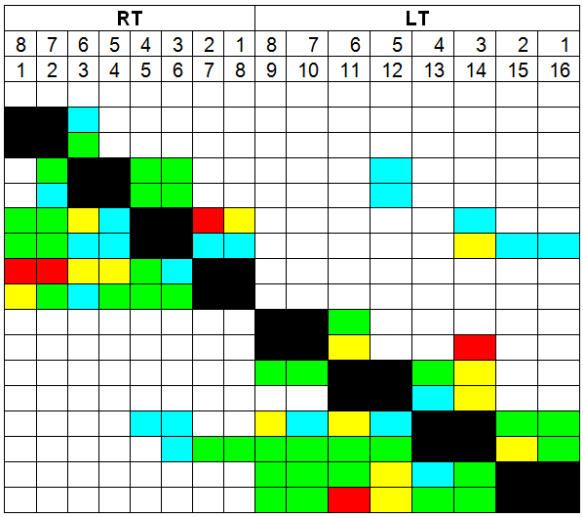


Figure 23: Patient with seizure onset is on the left temporal lobe (contacts 2-3).

Table 6 shows the congruence between presence of each condition (suppression, depression, facilitation and no-change) and the area and lobe of seizure onset. It also shows the congruence between areas stimulated to induce each condition and the area and lobe of seizure onset. In this small series, no specific condition appears to be a particularly good marker of seizure area or lobe.

Table 6. Congruence between each condition (or the area stimulated to induce each condition) with SO area.

Congruence between SO area and area stimulated to induce each condition								
	Pat 1	Pat 2	Pat 3	Pat 4	Pat 5	Pat 6	Pat 7	Average
Suppression	1/2 50%	1/2 50%	1/4 25%	1/5 20%	1/11 9%	1/4 25%	1/4 25%	29%
Depression	1/11 9%	0/3 0%	0/6 0%	1/9 11%	1/19 5%	1/8 12.5%	1/6 17%	8%
Facilitation	1/13 8%	1/5 20%	1/6 17%	1/7 14%	1/20 5%	1/10 10%	1/8 12.5%	12%
Similar	1/14 7%	1/4 25%	0/6 0%	1/8 12.5%	1/18 5.5%	1/8 12.5%	1/7 14%	11%
Congruence between SO area and area showing each condition								
	Pat 1	Pat 2	Pat 3	Pat 4	Pat 5	Pat 6	Pat 7	Average
Suppression	0/2 0%	1/3 33%	1/5 20%	1/14 9%	1/32 3%	0/9 0%	1/5 20%	12%
Depression	0/12 0%	1/5 20%	1/7 14%	0/21 0%	1/47 2%	2/14 8%	2/9 22%	12%
Facilitation	1/18 5.5%	1/6 17%	2/12 17%	0/17 0%	1/48 2%	2/21 9.5%	2/16 12.5%	9%
Similar	0/22 0%	3/11 27%	1/8 12.5%	0/15 0%	1/42 2%	1/16 6%	2/13 15%	7.5%

8. VISUAL ANALYSIS OF SUPPRESSION

The initial analysis was carried out visually in all 79 patients included in the study. During the course of this analysis it became clear that the visual analysis of depression or facilitation was impractical because it involved the comparison, in most cases, of responses to SPES and PPES of nearly similar amplitudes for several channels and many stimulations. For the 3,326 electrodes implanted, this analysis implied the comparison of SPES and PPES response amplitudes for each stimulation, resulting in over 11 million comparisons (namely, 3,326 squared comparisons, or 11,062,276 comparisons). For visual analysis, this was clearly impractical. However, visual analysis of suppression was visually possible because suppression is an on-off event, which is either present or absent. Presence of suppression was determined by visually identifying a flat PPES response in the presence of a non-flat SPES response.

For each patient, the regions showing suppression were compared with the seizure onset lobe and area, seizure onset type, neuropathology and surgical outcome.

For each stimulation, once the electrodes showing suppression were identified, the corresponding rectangle was filled in red in the checkerboard chart for the stimulation. Once this was carried out for all stimulations in each patient, the checkerboard chart for each patient was used to established if there was suppression at the seizure onset lobe (or when stimulating the seizure onset lobe). Presence or absence of suppression at the seizure onset lobe (or when stimulating the seizure onset lobe) was compiled for all patients, in order to calculate the tables below.

8.1 Relation between location of suppression and seizure onset lobe (Table 7).

Among the 79 patients initially included, the seizure onset lobe was identified in 73 patients, who were used to compile table 7. Sixty-two patients showed suppression. In 55 patients (55 out of 62 or 89%) suppression involved seizure onset lobe, either exclusively (31/62 or 50%) or in seizure onset lobe and other lobes (24/62 or 39%). Among the 31 patients who had suppression exclusively in the seizure onset lobe, sixteen patients had regional onset seizures, 12 had focal onset seizures and three patients had bilateral seizures (one independent and two generalised). Among the 24 patients with suppression in the seizure onset lobe and other lobes, 15 had focal seizures, eight patients had regional seizures, and one patient had focal and regional seizures. Among the seven patients showing suppression exclusively outside the seizure onset lobe, four patients had focal seizures, two patients had regional seizures, and one had independent focal and regional seizures. No suppression was seen in three patients with focal seizures and in eight with regional seizures.

In summary, suppression was mainly seen at the seizure onset lobe, either exclusively (42% of patients) or also in additional lobes (32% of patients). Suppression was seen exclusively outside seizure onset lobe in 10% of patients. Suppression was not seen in 15% of patients. The proportion of patients showing suppression exclusively in seizure onset was higher among those with regional onset (47%) compared to those with focal onset (35%) possibly reflecting more widespread pathophysiological abnormalities.

Comparison of the percentage of suppression observed exclusively in seizure onset lobe between focal and regional seizures: Forty two percent (42%) of patients with regional seizures and approximately one third (35%) of patients with focal seizures showed suppression exclusively in seizure onset lobe. However, the difference is not statistically significant (Z=0.986; p=0.32218).

Table 7. Relation between location of suppression and seizure onset lobe. F=focal; R=regional; S=suppression; SO=seizure onset.

	S exclusively in SO	S in SO lobe and other lobes	S exclusively outside SO lobe	NO S	Total patients
Focal SO	12 (35%)	15 (44%)	4 (12%)	3 (9%)	**34 (100%)**
Regional SO	16 (47%)	8(23.5%)	2 (6%)	8 (23.5%)	**34 (100%)**
F/R SO	0	1(50%)	1(50%)	0	**2 (100%)**
Bilateral	3 (100%)	0	0	0	**3 (100%)**
Total patients	**31 (42%)**	**24 (33%)**	**7 (10%)**	**11 (15%)**	**73 (100%)**

8.2 Distribution of suppression according to epilepsy syndrome.

8.2.1 Temporal lobe epilepsy (Table 8)

Among the 49 patients with temporal lobe epilepsy, suppression was seen in 40 (82%). Among these 40 patients with suppression, 20 showed suppression exclusively in the seizure onset lobe, 16 in the seizure onset lobe and other lobes, and four had suppression exclusively outside the seizure onset lobe. Among the 20 patients with suppression exclusively in the seizure onset lobe, 11 patients had focal and nine patients regional seizure onset. Among the 16 patients with suppression in seizure onset and other lobes, 12 had focal seizures, three had regional and one had independent focal (left mid-temporal lobe) and regional (lesion over the right posterior temporal lobe) seizures. All four patients with suppression exclusively outside seizure onset lobe had focal seizure onset. Three patients with focal and six patients with regional seizures had no suppression.

As in the whole population described in the previous section, suppression was mainly seen at the seizure onset lobe, either exclusively (41% of patients) or also in additional lobes (33% of patients). Suppression was seen exclusively outside seizure onset lobe in only 8% of patients. Suppression was not seen in nearly a fifth of patients (18%). Half of patients with regional seizure onset showed suppression exclusively in seizure onset lobe compared to over one third (37%) of patients with focal onset.

Comparison of the percentage of suppression observed exclusively in seizure onset lobe between focal and regional seizures: Half the patients with regional seizures and approximately one third (37%) of patients with focal seizures showed suppression exclusively in seizure onset lobe. However, the difference is not statistically significant (Z=0.907; p=0.36282).

Table 8. Distribution of suppression in patients with temporal lobe epilepsy using visual analysis. F=focal; R=regional; S=suppression; SO=seizure onset

	S exclusively in SO lobe	**S in SO lobe and other lobes**	**S exclusively outside SO lobe**	**No S**	**Total**
Focal SO	11 (37%)	12 (40%)	4 (13%)	3 (10%)	**30 (100%)**
Regional SO	9 (50%)	3 (17%)	0	6 (33%)	**18 (100%)**
F/R SO	0	1 (100%)	0	0	**1 (100%)**
Total	**20 (41%)**	**16 (33%)**	**4 (8%)**	**9 (18%)**	**49 (100%)**

8.2.2 Frontal lobe epilepsy (Table 9)

Suppression was found in all 10 frontal patients**,** exclusively in seizure onset lobe in three, in the seizure onset and in other lobes in three and exclusively outside seizure onset lobe in one. Among the three patients with suppression exclusively in seizure onset lobe, one patient had regional seizure onset and two patients showed bilateral seizure onset. Among the six patients with suppression in seizure onset and other lobes, three showed focal seizures and three showed regional seizures. The patient showing suppression exclusively outside the seizure onset lobe had regional seizures.

Although the number of patients is small, the majority of patients showed suppression in seizure onset lobe. The proportion of patients showing suppression exclusive in seizure onset lobe is only half of those showing suppression in seizures onset and other lobes. However, this difference though is not statistically significant (Z-Score is 1.348 and the p-value is 0.17702). As in the previous sections, the proportion of patients showing

suppression exclusively in seizure onset lobe was higher among those with regional onset than among those with focal seizure onset. However, this difference is not statistically significant (Z=1.348, p=0.1770).

Table 9. Distribution of suppression in patients with frontal lobe epilepsy using visual analysis. S=suppression; SO=seizure onset

	S exclusively in SO lobe	S in SO lobe and other lobes	S exclusively outside SO lobe	No S	Total
Focal SO	0	3 (100%)	0	0	**3 (100%)**
Regional SO	1 (20%)	3 (60%)	1 (20%)	0	**5 (100%)**
Bilateral SO	2 (100%)	0	0	0	**2 (100%)**
Total	**3 (30%)**	**6 (60%)**	**1 (10%)**	**0**	**10 (100%)**

8.2.3 Patients with independent seizure onset in two different lobes (Table 10)

Suppression was found in five out of six patients showing independent seizure onset in two different lobes. Four patients had independent frontal and temporal seizures and two patients had independent temporal and occipital seizures. All four patients with independent frontal and temporal seizures showed suppression exclusively in seizure onset lobes. Among these four patients, one patient had focal seizures, two patients had regional seizures and one patient had independent bi-frontal and left temporal seizures. Among the two patients with independent temporal and occipital seizures one had suppression exclusively outside seizure onset lobe and one did not show suppression.

Table 10. Distribution of suppression in patients with independent seizure onset in two different lobes using the visual analysis. S=suppression; SO=seizure onset

	S exclusively in SO lobe	S in SO lobe and other lobes	S exclusively outside SO lobe	No S	Total
Focal	1 (100%)	0	0	0	**1 (100%)**
Regional	2 (75%)	0	0	1 (25%)	**3 (100%)**
F/R	0	0	1 (100%)	0	**1 (100%)**
Bilateral	1 (100%)	0	0	0	**1 (100%)**
Total	**4 (67%)**	**0**	**1 (16.5%)**	**1 (16.5%)**	**6 (100%)**

Despite the limited number of patients it appears that two third of patients (67%) showed suppression exclusively in seizure onset lobe.

8.2.4 Patients with widespread seizure onset involving two lobes

All eight patients of this group had regional seizure onset. Four patients had suppression exclusively in seizure onset lobe. In two patients the lobes involved in seizure onset were the temporal and the parietal, in one patient the frontal and the parietal and in one the temporal and the occipital. Two patients, one with seizure onset over the frontal and temporal lobes and the other over the parietal and temporal lobes, had suppression in seizure onset lobe and other lobes. In one patient with seizure onset over the frontal and temporal lobes, suppression was seen exclusively outside seizure onset lobes. One patient with seizure onset over the frontal and parietal lobes, showed no suppression.

In summary half the patients with localised seizure onset involving two lobes showed suppression exclusively in seizure onset lobe and one quarter showed suppression in seizure onset and other lobes.

8.3. Relation between location of suppression and seizure onset area

Among the 34 patients with focal seizure onset, suppression was observed in 31 (91%). None of these patients had suppression exclusively in the focal seizure onset area (involving no more than three intracranial electrodes). Sixteen patients (47%) had focal seizures and suppression in seizure onset area and other areas. Among the 15 patients (44%) with suppression exclusively outside seizure onset area, four (27%) had suppression exclusively in the seizure onset lobe but outside the seizure onset area, four (27%) had suppression exclusively outside seizure onset lobe(s), six patients (40%) had suppression in seizure onset lobe and other lobes and one did not show suppression.

8.4. Relation between neuropathological findings and the distribution of suppression in seizure onset lobe (Table 11).

Among the 38 patients who underwent epilepsy surgery, neuropathology was available in 33 patients, and 14 patients showed suppression exclusively in seizure onset lobes.

Among these 14 patients, five had medial temporal sclerosis, four had focal cortical dysplasia (FCD), one had a dysembryoplastic neuroepithelial tumour (DNET), one had astrocytic changes, and in three no specific lesion was identified.

Among the 10 patients with suppression in seizure onset lobes and other lobes, three had medial temporal sclerosis (MTS), one patient had a DNET grade I, one showed astrocytosis, one showed evidence of hypothalamic hamartoma, one had a cystic lesion, one had, an ischaemic lesion, one had nodular heterotopia, and one had Rasmussen's syndrome.

In two patients, suppression was observed in lobes exclusively outside the seizure onset lobe. One patient had ganglioglioma and one displayed astrocytic changes.

Table 11. Relation between the distribution of suppression and the different type of neuropathological abnormalities. Epilepsy surgery patients are only considered using the visual analysis in both groups. S=suppression; SO=seizure onset; NS=Non-specific; NL=Non-lesional; N/P=Neuropathological; MTS=medial temporal sclerosis; DNET=dysembryoplastic neuroepithelial tumour.

N/P findings in pts with surgery	S exclusively in SO lobe	S in SO lobe and other lobes	S exclusively outside SO lobe	No S	Total no of pts
Normal /NS/NL	3	0	0	0	**3**
MTS	5	3	0	3	**11**
Tumours	2	3	2	1	**8**
Cystic lesions	0	1	0	1	**2**
Dysplastic lesions	4	0	0	1	**5**
Heterotopia	0	1	0	0	**1**
MTS + DNET	0	0	0	1	**1**
Ischaemic	0	1	0	0	**1**
Rasmussen syndrome	0	1	0	0	**1**
Total no of patients	**14**	**10**	**2**	**7**	**33**

Suppression was not recorded in seven patients, three had MTS, one had astrocytosis, one had FCD type IB, and one had no specific abnormalities. Suppression was not seen in one patient with MTS + DNET grade I.

8.5. Outcome and distribution of suppression in patients with MTS (Table 12)

The relation between the distribution of suppression in seizure onset lobe and the outcome in patients with medial temporal sclerosis (MTS) was examined. Thirteen patients with MTS underwent epilepsy surgery. Three had a follow-up shorter than nine months and were excluded from the analysis. Eight patients had a favourable outcome (grades I or II). Five of these patients had suppression exclusively in seizure onset lobe and three patients had no suppression. Among the two patients with poor outcome, one had suppression exclusively in the seizure onset lobe and one had suppression in seizure onset and other lobes.

Table 12. Outcome and distribution of suppression in patients with MTS using the visual analysis. S=suppression; SO=seizure onset; MTS=multiple temporal sclerosis; pts=patients. Stages I-IV according to Engel's.

Outcome	S exclusively in SO lobe	S in SO lobe and other lobes	S exclusively outside SO lobe	No S	Total no of pts with surgery
I+II	5 (63%)	0	0	3 (37%)	**8 (100%)**
III+IV	1(50%)	1(50%)	0	0	**2 (100%)**
Total MTS pts	**6 (60%)**	**1(10%)**	**0**	**3 (30%)**	**10 (100%)**

8.6. Surgical outcome (Table 13).

The surgical outcome was analysed in patients with more than nine months follow-up. Among the 26 patients who fulfilled the above criteria, 20 had favourable outcome. Six had focal seizures, 13 had regional seizures and one had independent focal and regional seizures. Among the six patients who had poor outcome (grades III and IV), three patients had focal seizures and three had regional seizures.

Table 13. Seizure outcome in patients operated. Stages I-IV according to Engel's classification. SO=seizure onset; F=focal; R=regional; pts=patients

	I+II	III+IV	Total epilepsy surgery pts
Focal SO	6 (67%)	3 (33%)	**9 (100%)**
Regional SO	13 (81%)	3 (19%)	**16 (100%)**
Independent F and R SO	1 (100%)	0	**1 (100%)**
Total pts - visual	**20 (77%)**	**6 (23%)**	**26 (100%)**

8.7 Resection of the areas showing suppression

Among the 38 patients who had epilepsy surgery, suppression was seen in 30 patients. Total resection of the area showing suppression was performed in 12 patients, partial resection in 12 patients and in six patients the area showing suppression was not resected.

8.8 Relation between surgical outcome and resection of areas showing suppression (Table 14).

Thirty-eight patients had epilepsy surgery. Twenty-six patients had a follow-up longer than nine months, 20 with a favourable outcome and six with a poor outcome. Among the 20 patients with favourable outcome, nine had the area showing suppression totally resected, five partially resected, in two patients the area showing suppression was not resected and four patients did not show suppression. Among the six patients who had a poor outcome, two had the area showing suppression partially resected, one totally resected, in two patients the area showing suppression was not resected, and one patient did not show suppression.

Table 14. Relation between surgical outcome and resection of areas showing suppression according to the visual analysis. Stages I-IV according to Engel's. S= suppression; TR=totally resected, PR=partially resected, NR=non- resected.

Outcome	TR	PR	NR	No S	**Total**
I+II	9 (45%)	5 (25%)	2 (10%)	4 (20%)	**20**
III+IV	1 (14%)	2 (33%)	2 (29%)	1 (14%)	**6**
Total	**10 (38%)**	**7 (27%)**	**4 (15%)**	**5 (20%)**	**26**

8.9 Relation between surgical outcome and resection of the areas of the stimulating electrodes showing suppression (Table 15)

Among the 38 patients who had epilepsy surgery, stimulating electrodes producing suppression were seen in 30 patients. The location of these stimulating electrodes was totally resected in 13 patients, partially resected in 12, and in five patients the location of the stimulating electrodes producing suppression was not resected.

Twenty patients had favourable outcome. In eight patients the location of the stimulating electrodes showing suppression was totally resected and in six partially resected. In two patients the location of the stimulating electrodes showing suppression was not resected and four patients did not show suppression.

Seven patients had poor outcome. In three patients the location of the stimulating electrodes showing suppression was totally resected, in two was partially resected, and in one the location of the stimulating electrodes showing suppression was not resected. One also patient did not show suppression.

Table 15. Relation between surgical outcome and resection of the areas of the stimulating electrodes showing suppression with the visual analysis. TR=totally resected; PR=partially resected; NR=non- resected.

Outcome	TR	PR	NR	No SRs	**Total**
I+II	8 (40%)	6 (30%)	2 (10%)	4 (20%)	**20**
III+IV	3 (50%)	1 (16.6 %)	1 (16.6%)	1 (16.6%)	**6**
Total	**11 (42%)**	**7 (27%)**	**3 (12%)**	**5 (19%)**	**26**

8.10 Relation between surgical outcome and resection of suppressed areas in the epileptic syndromes (Tables 16 and 17).

Temporal lobe epilepsy

Seventeen temporal patients had a follow-up longer than nine months. Suppression was seen in 13 patients. In eight patients the suppressed areas were totally resected, in four patients the suppressed areas were partially resected and in one patient no resection was observed. Among the 14 patients with favourable outcome (grades I and II), four had no suppression, three had their suppressed areas partially resected and seven totally resected. Among the three patients with poor outcome, one patient had no resection, one had their suppressed areas partially resected and one totally resected.

Statistical analysis: Statistical analysis **(**Fisher's exact test) was performed in temporal patients in order to show if there is any statistically significant association between surgical outcome and the resection of the suppressed area **(tables 16 and 17)**. Table 14

shows the association between surgical outcome (good v. poor) and the resection (total v. partial) of suppressed areas. Table 15 shows the association between surgical outcome (good v. poor) and the resection (total v. partial and non-resection) of suppressed areas. The two-tailed p-value in both cases is not statistically significant.

Table 16. Relation between surgical outcome and the resection of the suppressed area in temporal patients. Pts= patients; TR= total resection; PR=partial resection.

	Favourable outcome	Poor outcome	Total
Pts with TR	7	1	**8**
Pts with PR	3	1	**4**
Total	**10**	**2**	**12**

Table 17. Relation between surgical outcome and the resection of suppressed area in temporal patient. Pts= patients; TR=total resection; PR=partial resection and NR=non-resection

	Favourable outcome	Poor outcome	Total
Pts with TR	7	1	**8**
Pts with PR and pts with NR	3	2	**5**
Total	**10**	**3**	**13**

Frontal lobe epilepsy

Among the five frontal patients, four had a follow-up of more than nine months. The areas where suppression was seen were partially resected in all four patients. Favourable outcome was seen in two patients (grade II) and poor outcome in two patients (grade III and IV). Statistical analysis was not performed due to the limited number of patients.

Patients with independent seizure onset in two different lobes: Two patients had a follow-up of more than nine months. Both patients had independent frontal and temporal seizures. One patient had suppression in the frontal lobe and the resected areal involved the temporal lobe. Partial resection was seen in one patient with suppression over the right frontal and right temporal lobe. Good outcome (stage I) was seen in both patients.

Patients with widespread seizure onset involving two lobes Among the 4 patients with a follow-up of more than 9 months, 3 showed suppression. The suppressed area was totally resected in one patient, partially resected in one patient and not resected in one patient. The two patients with the total and partial resection had a good outcome (stage I). On the contrary the patient where the suppressed area was not resected and the patient who showed no suppression developed a poor outcome.

9. STUDY OF SUPPRESSION WITH VISUAL AND AUTOMATIC ANALYSES

For the reasons stated in methods (section *E.1.2. Automatic analysis*), a programme for automatic analysis was developed to study depression and facilitation. The programme was written to read files recorded with the Nervus system, which included 55 patients. Among these 55 patients, three did not have identifiable seizure. Consequently, the congruence between suppression features and seizure onset reported in this section was studied in the remaining 52 patients who showed identifiable seizure onset. Among these 52 patients, 49 showed seizures during telemetry and three patients did not suffer seizures during telemetry. The seizure onset lobe in these three patients was estimated by inducing their habitual seizures during functional stimulation. In the present section, we evaluate the automatic method by comparing results on suppression with those obtained from visual analysis of the same patients. The automatic method chosen was the comparison of SPES and PPES using the same latency and largest response. For the automatic analysis, a NALS threshold of 10 SD (±5 SD) was used.

9.1 Inter-rater agreement

The presence or absence of suppression exclusively in the seizure onset lobe was identified in all 52 patients on the checkerboard charts obtained with visual or automatic analyses. Table 18 shows the congruence on the location of suppression exclusively in seizure onset lobe between the two methods. Cohen's kappa coefficient for inter-rater agreement was 0.541, which is considered as "fairly good" agreement (Kirkwood and Sterne 2008).

Table 18. Inter-rater agreement and Cohen's Kappa coefficient. S=suppression; SO=seizure onset

		Automatic	**Automatic**
	S exclusively in SO lobe	**Yes**	**No**
Visual	**Yes**	18	3
Visual	**No**	9	22
Cohen's Kappa coefficient = 0.541			

9.2. Relation between location of suppression and seizure onset lobe

Visual analysis (Table 19): Among 46 patients with suppression, of which 39 had suppression in seizure onset lobe. In 21 of these 39 patients, suppression was noted exclusively in the seizure onset lobe. Eleven of these 21 patients had focal seizures, and eight had regional seizures and two had bilateral seizures. Eighteen had suppression in seizure onset lobe and in other lobes. Thirteen out of 18 patients had focal seizures and five had regional seizures. In seven of the 46 patients with suppression, suppression was observed exclusively outside seizure onset lobes. Four of these patients had focal seizures, two patients had regional and one had focal and regional seizures.

In conclusion, suppression was seen exclusively in the seizure onset lobe in approximately 40% of patients, regardless of whether seizure onset was focal or regional.

Table 19. ***Relation between location of suppression and seizure onset lobe according to visual analysis. S= suppression; SO = seizure onset; F=focal; R=regional***

	S exclusively in SO lobe	S in SO lobe and other lobes	S exclusively outside SO lobe	No S	Total patients
Focal SO	11 (37%)	13 (43%)	4 (13%)	2 (7%)	**30 (100%)**
Regional SO	8 (42%)	5 (26%)	2 (11%)	4 (21%)	**19 (100%)**
F/ R SO	0	0	1(100%)	0	**1 (100%)**
Bilateral SO	2 (100%)	0	0	0	**2 (100%)**
Total patients	**21 (40%)**	**18 (35%)**	**7 (13%)**	**6 (12%)**	**52 (100%)**

*Automatic analysis (Table 20). S*uppression was recorded in all patients with an identifiable seizure onset lobe. Among the 27 patients with suppression exclusively in seizure onset lobe, 15 had focal seizures, 10 had regional and two patients had bilateral seizures. Among the 14 patients with suppression in seizure onset lobe and other lobes, 10 had focal seizures and four had regional seizure onset. In 11 patients suppression was observed exclusively outside seizure onset lobe. Five patients had focal seizures, five had regional seizures and one patient had focal and regional seizure onset.

In conclusion, suppression was seen exclusively in the seizure onset lobe in approximately half the patients (52%), without a significant difference between focal (50%) and regional seizure onset patients (53%).

Table 20. ***Relation between location of suppression and seizure onset lobe according to automatic analysis. S= suppression; SO = seizure onset; F=Focal; R=Regional***

	S exclusively in SO lobe	S in SO lobe and other lobes	S exclusively outside SO lobe	No S	Total patients
Focal SO	15 (50%)	10 (33%)	5 (17%)	0	**30 (100%)**
Regional SO	10 (53%)	4 (21%)	5 (26%)	0	**19 (100%)**
F/R SO	0	0	1 (100%)	0	**1 (100%)**
Bilateral SO	2 (100%)	0	0	0	**2 (100%)**
Total patients	**27 (52%)**	**14 (27%)**	**11 (21%)**	**0**	**52 (100%)**

Comparison of visual and automatic analyses: Overall, suppression appeared more exclusively seen in seizure onset lobe with the automatic method (52%) than with visual analysis (40%). However, this difference is not significant (Z=1.18; p= 0.238).

9.3 Relation between location of stimulating electrodes producing suppression and seizure onset lobe

*Visual analysis (Table 21): S*uppression was recorded in 46 out of the 52 patients studied. Among the 24 patients with suppression exclusively in seizure onset lobe, 11 had focal seizures, 10 had regional seizures, two had bilateral seizures, and one had focal and regional seizures. Among the 18 patients with suppression in seizure onset lobe and other lobes, 14 had focal seizures, and four had regional seizure onset. In four patients suppression was observed in lobes exclusively outside seizure onset lobe. Three patients had focal seizures and one had regional seizures. Among the six patients with no suppression, two had focal seizures, and four had regional seizures.

In conclusion, the location of the stimulating electrodes producing suppression was observed exclusively in the seizure onset lobe in nearly half (46%) the patients. Approximately in one third of patients the location of the stimulating electrodes producing suppression was noted in the seizure onset lobe and other lobes as well.

Table 21. ***The distribution of stimulating electrodes producing suppression in seizure onset lobe according to the visual analysis. SE= stimulating electrodes producing suppression; SO=seizure onset; F=Focal; R=Regional***

	SE exclusively in SO lobe	SE in SO lobe and other lobes	SE exclusively outside SO lobe	No SE	Total patients
Focal SO	11 (37%)	14 (47%)	3 (10%)	2 (6%)	**30 (100%)**
Regional SO	10 (53%)	4 (21%)	1 (5%)	4 (21%)	**19 (100%)**
F/R SO	1 (100%)	0	0	0	**1 (100%)**
Bilateral SO	2 (100%)	0	0	0	**2 (100%)**
Total patients	**24 (46%)**	**18 (35%)**	**4 (8%)**	**6 (11%)**	**52 (100%)**

Comparison of the distribution of suppression and the distribution of the stimulating electrodes producing suppression with the visual method: The location of the stimulating electrodes producing suppression was observed exclusively in seizure onset lobe in nearly half (46%) the patients, as opposed to 40% of patients showing suppression exclusively in seizure onset lobe. The difference between both proportions is not statistically significant (Z=0.594, p= 0.5552).

9.4 Distribution of suppression according to epilepsy syndrome.

9.4.1. Temporal lobe epilepsy

Visual analysis (Table 22): Among the 35 temporal seizure onset patients suppression was seen in 31 (88.5%). Fourteen patients had suppression exclusively in seizure onset lobes. Ten had focal, and four had regional seizures. Among the 13 patients with suppression in seizure onset lobe and other lobes, 10 had focal and three had regional seizures. All four patients with suppression exclusively outside seizure onset lobe had focal seizures. Two patients with focal and regional seizure onset had no suppression.

In summary, forty percent (40%) of patients with seizure onset over the temporal lobe had suppression exclusively in seizure onset lobe, and a similar percentage (37%) showed suppression in seizure onset and other lobes.

Table 22. ***Distribution of suppression in temporal lobe epilepsy patients according to the visual analysis. S=suppression; SO=seizure onset***					
	S exclusively in SO lobe	**S in SO lobe and other lobes**	**S exclusively outside SO lobe**	**No S**	**Total patients**
Focal SO	10 (38%)	10 (38%)	4 (15%)	2 (8%)	**26 (100%)**
Regional SO	4 (44%)	3 (33%)	0	2 (23%)	**9 (100%)**
Total patients	**14 (40%)**	**13 (37%)**	**4 (11.5%)**	**4 (11.5%)**	**35 (100%)**

Automatic method (Table 23): Suppression was seen in all patients. Eighteen patients had suppression exclusively in seizure onset lobes. Fourteen had focal and four had regional seizures. Among the 12 patients with suppression in seizure onset lobe and other lobes, eight patients had focal and four had regional seizures. Four patients with focal and one with regional seizures had suppression exclusively outside seizure onset lobe.

In conclusion, suppression was seen exclusively in the seizure onset lobe in nearly half the patients (51%) with most of them (14/18 or 77%) having focal seizures.

Table 23. ***Distribution of suppression in temporal lobe epilepsy patients according to the automatic analysis. S=suppression; SO=seizure onset***					
	S exclusively in SO lobe	**S in SO lobe and other lobes**	**S exclusively outside SO lobe**	**No S**	**Total**
Focal SO	14 (54%)	8 (31%)	4 (15%)	0	**26 (100%)**
Regional SO	4 (44.5%)	4 (44.5%)	1 (11%)	0	**9 (100%)**
Total	**18 (51%)**	**12 (34%)**	**5 (14%)**	**0**	**35 (100%)**

Comparison of visual and automatic analyses: Suppression was observed exclusively in seizure onset lobe in approximately half (51%) the patients with automatic analysis and in 40% of patients with visual analysis. This difference is not significant (Z=0.96, p=0.33706).

9.4.2 Frontal lobe epilepsy

Visual analysis (Table 24): One patient with bilateral seizure onset had suppression exclusively in seizure onset lobe. Three patients with focal seizure onset and one with regional seizure onset showed suppression in seizure onset lobe and other lobes. One regional seizure onset patient had suppression exclusively outside seizure onset lobe.

Table 24. ***Distribution of suppression in frontal lobe patients according to the visual analysis. S=suppression; SO=seizure onset***					
	S exclusively in SO lobe	**S in SO lobe and other lobes**	**S exclusively outside SO lobe**	No S	**Total**
Focal SO	0	3 (100%)	0	0	**3 (100%)**
Regional SO	0	1 (50%)	1 (50%)	0	**2 (100%)**
Bilateral SO	1 (100%)	0	0	0	**1 (100%)**
Total	**1 (16%)**	**4 (67%)**	**1 (16%)**	**0**	**6 (100%)**

In conclusion, despite the limited number of patients in this section, suppression was seen in seizure onset lobe and other lobes (67%).

Automatic method (Table 25): Suppression was seen in all six frontal patients. One patient with bilateral seizure onset had suppression exclusively in seizure onset lobe. Two focal patients had suppression in seizure onset lobe and other lobes. Among the three patients with suppression exclusively outside seizure onset lobe, one had focal and two had regional seizures.

Table 25. ***Distribution of suppression in frontal lobe epilepsy patients according to the automatic analysis. S=suppression; SO=seizure onset***					
	S exclusively in SO lobe	**S in SO lobe and other lobes**	**S exclusively outside SO lobe**	**No S**	**Total**
Focal SO	0	2 (67%)	1 (33%)	0	**3 (100%)**
Regional SO	0	0	2 (100%)	0	**2 (100%)**
Bilateral SO	1 (100%)	0	0	0	**1 (100%)**
Total	**1 (17%)**	**2 (33%)**	**3 (50%)**	**0**	**6 (100%)**

In summary, although patient numbers are small, it appears that half of patients showed suppression exclusively outside seizure onset lobe. Only one fifth (17%) of the patients showed suppression exclusively in seizure onset lobe.

Comparison of visual and automatic analyses: With visual analysis 67% of patients showed suppression in seizure onset lobe and other lobes as opposed to 33% with the automatic analysis. However, the difference is not significant ($Z= 1.155$; $p=0.25014$).

9.4.3 Patients with independent seizure onset in different lobes

Visual analysis (Table 26): Among the five patients with independent seizure onset in different lobes, frontal and temporal lobes were involved in three patients. One had focal seizure onset, one had regional seizure onset and one had bilateral independent (frontal and temporal) seizure onsets. All three patients showed suppression exclusively in seizure onset lobes. In the remaining two, patients the temporal and occipital lobes were involved. One showed suppression exclusively outside seizure onset lobes and one showed no suppression responses.

Table 26. ***Distribution of suppression in epilepsy patients with independent seizure onset in two different lobes according to the visual analysis. S=suppression; SO=seizure onset***

	S exclusively in SO lobe	**S in SO lobe and other lobes**	**S exclusively outside SO lobe**	**No S**	**Total**
Focal SO	1 (100%)	0	0	0	**1 (100%)**
Regional SO	1 (50%)	0	0	1 (50%)	**2 (100%)**
F/R SO	0	0	1 (100%)	0	**1 (100%)**
Bilateral SO	1 (100%)	0	0	0	**1 (100%)**
Total	**3 (60%)**	**0**	**1 (20%)**	**1 (20%)**	**5 (100%)**

Despite the limited number of patients it appears that suppression was observed exclusively in seizure onset lobe in most patients (60%).

Automatic method (Table 27): In one of the two patients with regional seizures, suppression was observed in both seizure onset lobes. In one of the two patients with regional seizure onset and the patient with bilateral seizure onset suppression was observed in one of the two seizure onset lobes (part of seizure onset lobe).

Although the number of patients is small, suppression was observed exclusively in seizure onset lobe in 80% of patients.

Comparison of visual and automatic analyses: Four patients out of five (80%) showed suppression exclusively in seizure onset lobe with the automatic method as opposed to

60% when the visual method was used. The difference is not significant at p <.05 (Z-Score is 0.69 and p-value is 0.4902), probably because of the small number of patients.

Table 27. ***Distribution of suppression in patients with independent seizure onset in two different lobes according to automatic analysis. S=suppression; SO=seizure onset; F=focal; R=regional***

	S exclusively in SO lobe	S in SO lobe and other lobes	S exclusively outside SO lobe	No S	Total
Focal SO	1 (100%)	0	0	0	**1 (100%)**
Regional SO	2 (100%)	0	0	0	**2 (100%)**
F/R SO	0	0	1 (100%)	0	**1 (100%)**
Bilateral	1 (100%)	0	0	0	**1 (100%)**
Total	**4 (80%)**	**0**	**1 (20%)**	**0**	**5 (100%)**

9.4.4. Patients with widespread seizure onset involving two lobes

Visual analysis: Among the six patients with regional seizure onset involving two lobes, three (50%) had suppression exclusively in seizure onset lobe. In one patient, the lobes involved in seizure onset were the temporal and the parietal, in one patient were the frontal and the parietal and in one the temporal and the occipital lobes. One patient with seizure onset over the parietal and temporal lobes showed suppression in seizure onset lobes and other lobes. In one patient with seizure onset over the frontal and temporal lobes, suppression was seen exclusively outside seizure onset lobes. In one patient with seizure onset over the frontal and parietal lobes no suppression was seen.

Overall half the patients (three out of six or 50%) with widespread seizure onset involving two lobes showed suppression exclusively in seizure onset lobe.

Automatic method: Suppression was seen in all six patients. Four patients showed suppression exclusively in seizure onset lobe. Among the four patients with suppression exclusively in seizure onset lobes, only one patient showed suppression over both seizure onset lobes. In the remaining three patients, suppression was observed in one of the two seizure onset lobes. Two patients had suppression exclusively outside seizure onset lobes.

Although the number of patients is small, it seems that two third of patients (4/6 or 67%) with widespread seizure onset involving two lobes, showed suppression exclusively in seizure onset lobe.

Comparison of visual and automatic analyses: Two thirds of patients showed suppression exclusively in seizure onset lobes with the automatic method as opposed to half the patients with the visual method. The difference is not significant at $p < .05$ (Z-Score is 0.586 and p-value is 0.5552), possibly due to the small patient numbers.

9.5. Conclusion

The automatic method is effective in detecting suppression, with results comparable to those of visual analysis. In fact no difference was shown between the two methods even in the context of the different epilepsy syndromes studied.

10. STUDY OF DEPRESSION, SUPPRESSION AND FACILITATION

As already explained in the methods, (section *E.1.2. Automatic analysis*) a programme was developed for automatic analysis of depression and facilitation. The programme was designed to read files recorded with the Nervus system, which included 55 patients. Automatic analysis used the method, described as "same latency and largest response" in the methods section. In essence, this method compares the largest amplitude of responses to SPES to the amplitude of responses of PPES measured approximately at the same latency (within a window of ±25 ms) as the SPES responses. Since responses showed a widespread distribution, the highest threshold of five NALS (10 standard deviations of the baseline noise level) was used to identify responses.

In three out of 55 patients, seizure onset was not identified and, consequently, the analysis was performed in 52 patients. Three out of the 52 patients did not have seizures during the telemetry period and, in these three patients, seizure onset was estimated by induction of the patients' habitual seizures with functional stimulation.

10.1. Relation to seizure onset lobe

10.1.1. All 52 patients

Table 28 shows for all 52 patients, the incidence of depression, suppression, facilitation and similar responses seen exclusively in the seizure onset lobe, in the seizure onset lobe and other lobes and exclusively outside seizure onset lobes. Suppression was the response type most frequently seen exclusively at the seizure onset type, even though it was the response type less frequently seen overall (not present in 33% of patients).

Table 28. Presence of suppression, depression, similar responses and facilitation at the seizure onset lobe and in other lobes. SO= seizure onset.

Response type	Exclusively in SO lobe	In SO lobe and other lobes	Exclusively outside SO lobe	No responses	Total
Suppression	20 (38%)	7 (13%)	8 (15%)	17 (33%)	**52**
Depression	10 (19%)	37 (71%)	5 (10%)	0	**52**
Similar	17 (33%)	28 (54%)	7 (13%)	0	**52**
Facilitation	13 (25%)	31(60%)	8 (15%)	0	**52**

10.1.2. Patients with focal seizure onset

For the 30 patients with focal seizure onset, table 29 shows the incidence of depression, suppression, facilitation and similar responses exclusively seen in the seizure onset lobe, in the seizure onset lobe and other lobes, and exclusively outside seizure onset lobes. Among the patients that showed changes between and SPES and PPES responses, suppression was the response most specifically associated with seizure onset lobe (seen exclusively in seizure onset in 37% of patients), despite the fact that it was the response type less commonly seen (not present in as many as 33% of patients).

Table 29. Relation of suppression, depression, similar and facilitation to seizure onset lobe in focal patients (10 SD). SO= seizure onset; SD=standard deviation

Response type	Exclusively in SO lobe	In SO lobe and other lobes	Exclusively outside SO lobe	NO response	Total
Suppression	11 (37%)	5 (17%)	4 (13%)	10 (33%)	**30**
Depression	5 (17%)	24 (80%)	1 (3%)	0	**30**
Similar	11 (37%)	16 (53%)	3 (10%)	0	**30**
Facilitation	7 (23%)	17 (57%)	5 (17%)	1(3%)	**30**

10.1.2.1 Patients with temporal focal seizure onset

For the 26 patients showing focal seizure onset restricted to the temporal lobe, table 30 shows the incidence of depression, suppression, facilitation and similar responses exclusively seen in the seizure onset lobe, in the seizure onset lobe and other lobes, and exclusively outside seizure onset lobes. Overall, suppression was the response type less commonly seen (not observed in 38%). By contrast, depression and no-change responses were seen in all patients and facilitation was seen in all but one patient (4%). However, suppression was the response type most frequently seen exclusively in seizure onset lobe (in 38%). Nevertheless, the proportions of suppression and depression found exclusively in seizure onset lobe were not statistically different (Z=1.53; p=0.126).

Table 30. Relation of suppression, depression and facilitation with seizure onset lobe in 26 temporal focal patients (10 SD). SO= seizure onset; SD=standard deviation

Response type	**Exclusively in SO lobe**	**In SO lobe and other lobes**	**Exclusively outside SO lobe**	**No response**	**Total**
Suppression	10 (38%)	3 (12%)	3 (12%)	10 (38%)	**26**
Depression	5 (19%)	20 (77%)	1 (4%)	0	**26**
Similar	10 (38%)	13 (50%)	3 (12%)	0	**26**
Facilitation	6 (23%)	14 (54%)	5 (19%)	1(4%)	**26**

10.1.2.2. Patients with frontal focal seizure onset

All three frontal patients with focal seizure onset showed facilitation and depression in seizure onset lobe and in other lobes. In two patients suppression was observed in seizure onset lobe and other lobes, and in one patient exclusively outside seizure onset lobe.

10.1.2.3. Patients with independent seizure onset in two different lobes

Among the two patients with independent seizure onset in two different lobes, one showed facilitation exclusively in seizure onset lobe and one in seizure onset lobe and other lobes. In both patients, depression was observed in seizure onset lobe and other lobes. One patient showed suppression exclusively in seizure onset lobe and one exclusively outside seizure onset lobe.

10.1.3. Regional seizure onset patients

All 17 patients with regional seizure onset showed depression and facilitation. Suppression was observed in twelve patients. As in patients with focal seizure onset, suppression was the response type most commonly seen exclusively in seizure onset lobe (47% of patients) despite being the less commonly seen response overall (not seen in 29% of patients). By contrast, all patients showed the remaining response types.

Table. 31. Relation of facilitation, depression, suppression and similar with SO lobe (10 SD). SD=standard deviation; SO=seizure onset lobe.

Response type	**Exclusively in SO lobe**	**In SO lobe and other than SO lobe**	**Exclusively outside SO lobe**	**No response**	**Total**
Suppression	8 (47%)	2 (12%)	2 (12%)	5 (29%)	**17**
Depression	4 (23.5%)	9 (53%)	4 (23.5%)	0	**17**
Similar	6 (35%)	8 (47%)	3 (18%)	0	**17**
Facilitation	5 (29%)	10 (59%)	2 (12%)	0	**17**

10.1.3.1. Patients with temporal onset

As in the previous groups, suppression was the response type most commonly seen in seizure onset lobe (Table 32). Nearly half the patients with temporal regional seizure onset (4 out of 9 or 44%) showed suppression exclusively in seizure onset lobe, despite being the only response type not observed at all in some patients.

Table. 32. Relation of facilitation, depression, suppression and similar to seizure onset lobe in 9 temporal regional patients					
Response type	**Exclusively in SO lobe**	**In SO lobe and other than SO lobe**	**Exclusively outside SO lobe**	**No responses**	**Total**
Suppression	4 (44%)	2 (22.5%)	1(11%)	2 (22.5%)	**9**
Depression	1(11%)	6 (67%)	2 (22%)	0	**9**
Similar	2 (22%)	6 (67%)	1(11%)	0	**9**
Facilitation	2 (22%)	6 (67%)	1(11%)	0	**9**

10.1.3.2. Patients with frontal seizure onset

There were only two frontal patients with regional seizure onset. One patient had suppression exclusively outside seizure onset lobe. The same patient showed depression, facilitation and similar responses in seizure onset lobe and other lobes. The second patient did not show suppression. Depression, facilitation and similar responses were observed exclusively outside seizure onset lobe.

10.1.3.3. Patients with independent regional seizure onset in two different lobes

Two patients with independent seizure onset in two different lobes were observed. In the first patient seizures arise from the left temporal and the left occipital lobe. Similar, facilitated and depressed responses were observed exclusively in seizure onset lobe. No suppressed responses were observed. In the second patient seizures arise from the left frontal and temporal lobes. All responses (facilitation, depression, suppression and similar) were observed exclusively in seizure onset lobe.

10.1.3.4. Patients with widespread seizure onset involving two lobes

Four patients with widespread seizure onset involving two lobes were observed. In the first patient, seizures arose from the left temporo-parietal lobes. Suppression and similar

responses were observed exclusively in seizure onset lobe, and depression and facilitation in seizure onset lobe and other lobes. The seizures of two patients arose from the right fronto-parietal lobes. In one of these two patients, depression, facilitation and similar responses were observed in seizure onset lobe and other lobes. No suppression was noted in this patient. In the second patient with seizures from the right fronto-parietal lobes, suppression was observed exclusively in seizure onset lobe, facilitation in seizure onset lobe and other lobes, and depressed and similar responses exclusively outside seizure onset lobes. In the fourth patient, seizures arose from the right temporo-occipital lobes. All responses were observed exclusively in seizure onset lobe.

10.2. Relation between surgical outcome and resection of areas showing depression

Among the 24 patients with epilepsy surgery, 12 had a follow-up longer than nine months. Nine patients out of twelve showed a favourable outcome (stages 1 and 2 according to Engel's) and three had a poor outcome. In six patients with favourable outcome, the area showing depression was partially resected, in two totally resected and in one no depression responses were observed. In two patients with poor outcome the area showing depression was partially resected, and in one no depression was seen.

10.3. Relation between surgical outcome and resection of areas showing facilitation

Among the 12 patients with epilepsy surgery and a follow-up longer than nine months, nine had favourable outcome and three had a poor outcome. In three out of nine patients with good outcome, the area showing facilitation was totally resected, in five was partially resected and in one patient no facilitation was observed. In two of the patients with poor outcome the area showing facilitation was partially resected and in one not resected

DISCUSSION

DISCUSSION

After the comparison between the responses elicited by first and second stimuli of the paired pulse four different conditions emerged: a) no change between the two responses (similar responses), b) facilitation, c) depression and d) suppression. These conditions were compared to seizure onset lobe and surgical outcome.

Four pilot studies were initially carried out to estimate the best experimental parameters and the effects limiting methodological factors. In essence, the pilot studies suggested that: a) 200 ms PPES inter-stimulus interval was the most likely to induce the above responses; b) The error between identical amplitude measurements (due to aliasing of the stimulus artefact and other factors) is 4.8%; c) The artefact correction routine used by the software did not induce significant differences on amplitude measurements; and d) None of the responses described above appeared to be better markers *a priori* for the epileptogenic area.

Consequently, larger studies were carried out with 200 msec PPES interstimulus intervals to investigate the utility of suppression, depression and facilitation as markers for epileptogenic cortex and, by implication, their use during presurgical assessment of focal epilepsy. The presence of each response type in the seizure onset lobe was estimated, and the relation between seizure outcome and removal of each response type investigated.

The following interesting results are worth commenting:

1) Suppression, depression and facilitation were observed in the seizure onset lobe and in other lobes.

2) Suppression was seen exclusively in the seizure onset lobe (or when stimulating exclusively the seizure onset lobe) in 31-52% of patient groups larger than 10. By contrast, depression was seen exclusively in seizure lobe in only 17-23.5% of patients and facilitation in 23-29 %. This suggests that suppression is more **sensitive** to detect the epileptogenic lobe than the depression and facilitation.

3) Suppression was seen in seizure onset lobe and in other lobes in 11.5-17% of patients. However, depression and facilitation were seen in seizure onset lobe and in other lobes in 53-80% and 54-60% of patients respectively, suggesting that suppression is more **specific** than depression or facilitation.

4) The association between seizure onset lobe and the distribution of suppression was observed independently of the methods of analysis applied. Although the proportions of patients showing suppression were higher with the automatic analysis, the differences were not statistically significant. Automatic analysis would be necessary if these methods were to have clinical applications, as the number of comparisons required would be impractical for visual analysis.

5) Resection of the areas showing suppression was not a reliable predictor of surgical outcome, possibly due to the relatively low sensitivity and specificity of suppression.

As suggested in the introduction, depression (and its most extreme case, suppression) probably represents increased inhibition and facilitation may represents excitation or reduced inhibition. Our findings of a rather ubiquitous distribution of suppression, depression and facilitation probably reflect the fact that inhibition and excitation are rather generic mechanisms widely distributed throughout the cortex. We have found that suppression, possible representing the most extreme form of inhibition, is not only present in the epileptogenic lobe, but is the most sensitive and specific response of those studied. This suggest that the epileptogenic lobe may contain powerful inhibition, and even more so than other lobes. The congruence with suppression is much lower for the epileptogenic area than for the epileptogenic lobe, suggesting that most of inhibition in the epileptogenic lobe is outside the epileptogenic area.

1. Inhibition in epilepsy

1.1. Depression

Our findings would be in agreement with findings from other human studies especially that performed by Uruno et al (1995). Uruno and colleagues used the PPES protocol in the perforant pathway recording from the dentate gyrus. Slices from patients with temporal lobe epilepsy were examined in order to cast some light into the mechanisms of paired pulse depression. Inhibition was expressed as the paired pulse ratio defined as the population spike amplitude of the second response (PS2) to the population spike amplitude of the first response (PS1). Two groups of patients were seen according to the magnitude of the highest paired pulse ratio: a) a strong group (PS2/PS1=0.12), and b) a weak group (PS2/PS1=0.68). Pharmacological blockade by the use of bicuculline and baclofen led to reduction of paired pulse depression and occasionally paired pulse facilitation. Findings showed: a) less inhibition in the weak group compared with the strong group; b) reduction of feedback inhibition in the strong group by pharmacological means; and c) paired pulse facilitation once inhibition was decreased. They concluded that inhibition is not always lost in epilepsy patients.

Evidence of increased inhibition was also seen in animal studies. However all these animal studies are experimental studies in that specific subpopulation of cells was stimulated and used for recording. Moreover, different models of epilepsy (kindling, kainic acid, or status epilepticus) have been used. Therefore, there is uncertainty regarding the relevance of results from animal studies and whether they could be applied to human focal epilepsy. In most studies, *in vivo* or *in vitro* rat models have been used after stimulation of the perforant path (Tuff 1983 a, Tuff 1983 b, King et al 1985, Maru and Goddard 1987 c, De Jonge and Racine 1987 Stringer and Lothman 1989, Davies et al 1990, Sloviter 1991a, Zhao and Leung 1992, Milgram et al 1994 and Haas et al 1996). One of the most important studies was performed by Tuff et al (Tuff 1983a). They used the kindling model of epilepsy in rats to detect enhanced paired pulse depression in the dentate gyrus after stimulation of the perforant path. Two interesting results were observed: a) the latency to the afterdischarge (AD) onset after kindling stimulation was increased and b) paired pulse depression was accentuated by GABA agonists and

attenuated by GABA antagonists. Therefore it was inferred that increased paired pulse depression reflects a prolonged GABA mediated post-excitation inhibition. This would also suggest the presence of an intact inhibitory mechanism. The same group in another study (Tuff 1983 b) estimated the number of GABA and associated benzodiazepines receptors in specific brain areas. This was performed by measuring GABA and muscimol binding, following kindling in rats. They observed that: a) the number of GABA receptors was not modified, and b) the number of benzodiazepine receptors following kindling was increased in amygdala and hippocampus. They inferred that since GABA transmission is enhanced by benzodiazepine receptors, this finding would reflect enhanced inhibition.

King and colleagues used the kindling model of epilepsy in rats (King et al 1985). The lateral entorhinal cortex was stimulated and the dentate gyrus was recorded. Strong evidence in favour of enhanced inhibition was seen. They observed that: a) the current intensity for the standard-size population spike to be evoked was increased; and b) paired pulse inhibition was greater in the kindled slices of the dentate gyrus. Taking into account that: a) there were no differences in excitatory postsynaptic potentials (EPSPs) between control and kindled slices; and b) paired pulse inhibition was reduced by GABA antagonists the authors concluded that the above findings expressed enhanced synaptic inhibition rather than decreased excitability.

1.2. Suppression

In our series, suppression showed a stronger association with seizure onset lobe than depression or facilitation. Suppression is an extreme depression and probably represents increased inhibition. Increased inhibition was seen in a few human (Wilson et al 1998, Uruno et al 1995) and animal studies (Steffensen and Henriksen 1991, Chowdhury et al 1996, Haas et al 1996 and Xu et al 2009). The study by Wilson's and colleagues (1998) is of paramount importance as it is one of the few studies with clear evidence of increased inhibition in patients with intractable temporal lobe epilepsy. All twenty patients were candidates for anterior temporal lobe resection. Paired pulse electrical stimulation was performed in the epileptogenic and the non-epileptogenic hippocampus. After such a comparison the following results were emerged: a) increased inhibition was greater in the epileptogenic rather in the non-epileptogenic side; b) increased inhibition was greater in

the epileptogenic perforant path as opposed to the non-epileptogenic perforant path; and c) increased inhibition was greater in the epileptogenic hippocampus mainly after stimulation of the perforant path, rather than after stimulation of the intrinsic associational pathways.

Of the animal studies Steffensen and Henriksen (1991) study was very interesting because provided evidence for the anatomical substrate of inhibition at a molecular level. Baclofen ($GABA_B$ agonist), bicuculline ($GABA_A$ antagonist) and phaclofen ($GABA_B$ antagonist) were used to study the mechanisms involved in inhibition. They applied the paired pulse protocol in the perforant path and the Shaffer collateral in rats, recording respectively from the dentate gyrus (DG) and the CA1 pyramidal cells. They observed that: a) baclofen enhanced the amplitude of the population spikes in the DG but in CA1 a decrement was noted; b) bicuculline increased the amplitude in both the DG and the CA1 cells; and c) phaclofen reversed the action of baclofen. Therefore they concluded that *"feed-back and feed-forward inhibition is mediated by postsynaptic $GABA_A$ receptors located on the somata of DG cells and CA1 pyramidal cells and by presynaptic $GABA_B$ receptors located on the synaptic terminals of GABAergic interneurons. CA1 differs from the dentate by the incorporation of a $GABA_B$ receptor located postsynaptically on CA1 cells"*.

In accordance with the above, Chowdhury et al (1996) used the PPES to gain more insight into the role of $GABA_B$ receptors in cat's motor cortex. A specific $GABA_B$ antagonist attenuated while baclofen accentuated paired pulse inhibition. They inferred that the inhibition of the spike response by the 2nd stimulus is possible due to depression to the excitatory potential being depressed and that $GABA_B$ receptors regulate such excitability in cat's motor cortex.

Xu et al (2009) though, in an interesting experiment with *in vitro* hippocampal slices preparations, explored the mechanism of inhibition of the response to be elicited from the 2nd pulse of the paired pulse electrical stimulation. They suggested that paired pulse depression (and suppression) is mediated from the activation of $GABA_C$ receptors as a $GABA_C$ antagonist (TMPA) blocked paired pulse depression without affecting $GABA_A$ or $GABA_B$ receptors.

1.3 Reduced inhibition

Animal studies from other authors further support the presence of increased excitation in epilepsy (Kamphuis et al 1988, Kapur et al 1989, Shirasaka and Wasterlain 1994, Emori et al 1997, Naylor 2002, Naylor and Wasterlain 2005, Ikeda et al 1998, Sloviter 1991, Fueta et al 1998 and Schmidt et al 2006). Kamphuis and colleagues using the kindling model of epilepsy in rats noted a progressive disinhibition leading to excitation when recording from CA1 pyramidal neurons. According to the authors this excitation was due to the reduced inhibition and threshold of the pyramidal neurons. On the other hand Naylor (2002) used the status epilepticus model of epilepsy in rats stimulating the perforant pathway. The loss of inhibition that noted was due to the reduced sensitivity of the benzodiazepine receptor affecting GABAergic function. This was interpreted as an early transition to status. Wasterlain and Naylor (2005), in an analogous study, observed that bicuculline caused similar loss of inhibition supporting the conclusion from the previous studies. Therefore different forms of hyperexcitability were noted both increased inhibition and facilitation.

1.4. Surround inhibition

Our findings suggest the absence of inhibition at the seizure onset area and presence of inhibition around the seizure onset area. Among patients with focal seizure onset, no patient had suppression exclusively in seizure onset area whereas 80% (table 19 of the results) showed suppression in seizure onset lobe either exclusively or in addition to other lobes. In addition, only 47% of patients showed suppression in seizure onset area and/or other areas. In summary, there was a higher association between suppression and seizure onset lobe than between suppression and seizure onset area. The full extent of this difference may be better appreciated when the percentage of suppression found exclusively in seizure onset lobe or area is taken into account. This percentage was found to be 37% when seizure onset lobe was considered and 0% in the case of seizure onset area. At the same time, the percentage of patients with suppression exclusively outside seizure onset area was increased compared to exclusively outside seizure onset lobe (15 out of 34 focal onset patients or 44%). Moreover, nearly one third of these patients (four out of 15 patients or 27%) showed suppression exclusively outside seizure onset area but inside seizure onset lobe. As mentioned above, this would support the presence of

inhibition around seizure focus. The presence of surround inhibition has recently been suggested on the basis of single cell recordings from our group (Alarcon et al, 2012). The percentage of inhibition is also supported by our finding that among the patients with focal onset, none showed suppression exclusively at seizure onset area whereas nearly half (47%) the patients showed suppression at seizure onset area and other areas.

Changes in surround inhibition may be highly relevant to epileptogenesis. The role of inhibition on synchronization was shown in a very interesting study performed by Isokawa-Akesson et al. (1989). Single and paired pulse electrical stimulation was used in hippocampal neurons of patients with temporal lobe epilepsy. It was found that the duration of inhibition in synchronously firing epileptic neurons is longer than the duration of inhibition in non-synchronously firing neurons. This finding according to the authors would imply that not only the inhibitory system would be intact and functional but also that more recurrent inhibitory circuits could be recruited if pyramidal neurons fire in synchrony. The above study emphasizes the important role of inhibition in producing synchronous epileptiform discharges just as inhibitory mechanisms are the basis of synchronization even in the normal brain (Ackermann et al 1964).

2. Differences according to epilepsy syndrome

In our patient sample, the main differences lie between temporal and frontal seizure onset patients. In temporal lobe patients, the highest percentage of suppression was seen exclusively in seizure onset lobe (41% with visual method and 51% with automatic method). By contrast, in frontal patients, the highest percentage of suppression was seen either at seizure onset lobe and other lobes (67% with the visual method), or exclusively outside seizure onset lobe (50% with the automatic). This difference between temporal and frontal onset patients may be due to the profuse connections between frontal lobes and other lobes (Lacruz et al, 2007) but the interpretation of these results is difficult because of the limited number of frontal patients (in fact the difference was not statistically significant).

3. Focal versus regional seizure onset

We did not find any statistical differences between patients with focal or regional seizure onsets, probably due to the fact that suppression and other synaptic changes appear not to be very specific to the seizure onset area but may show a wider distribution throughout the seizure onset lobe.

4. Surgical outcome

An intriguing question that has emerged is that, despite the association between suppression and seizure onset lobe, resection of the lobe where suppression was seen, was not found to be a reliable marker for surgery outcome. There was no statistically significant association between surgical outcome and removal of the regions showing suppression.

One explanation might be that few patients showed suppression in seizure onset area. This effect was observed especially in patients with focal seizure onset. In fact, as described above (section 1.4) no patient with focal seizure onset showed suppression exclusively in the seizure onset area. Half patients (47%) showed suppression inside seizure onset area and other areas and approximately the remaining half (43%) showed suppression exclusively outside seizure onset area. The presence of a higher association between suppression and seizure onset lobe compared to seizure onset area would imply that the area of suppression (inhibition) is located in the epileptogenic lobe but outside the seizure onset area. Therefore removal of areas with suppression may not be associated with better outcome, as it may not necessarily imply removal of the epileptogenic areas.

5. Methodological considerations

All methods used showed essentially similar results with regard to the association of suppression and seizure onset lobe (compare tables 19 and 20). Statistical analysis showed no significant difference between the two methods.

Part of the congruence between seizure onset lobe and distribution of responses may be due to the fact that that electrode implantation is necessarily bias towards the epileptogenic lobe. In our focal patients, an average of 58% of electrodes was implanted in the seizure onset lobe. So, if suppression was not related to the epileptogenic lobe, there may appear to be an association between suppression and the epileptogenic lobe due to the fact that there are more electrodes there. However, this is unlikely to be the only explanation, as there was no suppression at the seizure onset area, which is precisely the most targeted location for electrode implantation.

6. Stimulating electrodes producing suppression

Results were not different when the areas showing suppression and the areas stimulated in order to show suppression were used. This is to be expected as the regions underneath the stimulating electrodes are necessarily connected with the regions showing suppression.

7. Future avenues

First, further animal and human studies are necessary in order to clarify the role of inhibition and excitation in epileptic seizures.

Second, the present study is essentially qualitative, as we have studied presence or absence of the different response types. Quantitative studies, exploring the effect on different magnitudes of depression and facilitation may be useful to quantify features of the epileptogenic areas or lobes. This may be particularly relevant for facilitation, as the strongest depression (suppression) has been included in this study. There may be other parameters that could improve localisation of seizure onset and predict the surgical outcome, such as the duration or integral of the response elicited from the second pulse of the paired pulse.

Finally, PPES may help evaluate the effects of antiepileptic drugs and how these may affect excitability of the epileptogenic cortex. In this respect, paired-pulse magnetic stimulation may prove useful in monitoring the effects and dose of antiepileptic drugs.

CONCLUSION

CONCLUSION

The purpose of this study was the identification of synaptic changes related to epileptogenesis in patients investigated with intracranial recordings during presurgical assessment. Paired pulse electrical stimulation was used. After comparing response amplitudes elicited by the first and second pulse, four different conditions emerged: a) no change, b) facilitation, c) depression and d) suppression. To define the best marker for the identification of the epileptogenic cortex, the location of such conditions were compared to location of the seizure onset lobe and their removal correlated with surgical outcome.

Suppression appears to be a better marker in detecting the epileptogenic lobe than depression and facilitation because it is seen exclusively in the seizure onset lobe on a greater percentage of patients. The association between seizure onset lobe and the distribution of suppression was similar for visual and automatic methods. Removal of areas with suppression was not a reliable predictor of surgical outcome.

In patients with focal seizure onset suppression, was seen in the area surrounding the focus as no patient showed suppression exclusively in seizure onset area. This suggests the presence of inhibition around the seizure focus in the epileptogenic lobe, which may play a protective role during the interictal state.

SUMMARY

SUMMARY

The purpose of this study was the identification of synaptic changes related to epileptogenesis in patients investigated with intracranial recordings during presurgical assessment. Paired pulse electrical stimulation was performed. After comparing the response amplitudes elicited by the first and second pulse, four different modalities were emerged: a) no change, b) facilitation, c) depression and d) suppression. Suppression appears proved to be a better marker in detecting the epileptogenic lobe because it was seen exclusively in the seizure onset lobe on a greater percentage of patients. Moreover in patients with focal seizure onset it was seen in the area surrounding the focus as no patient showed suppression exclusively in seizure onset area.

APPENDIX I

APPENDIX 1

In this appendix examples of suppression for each patient are shown. The patients with inconclusive SO but with suppression are included. Patients 20, 37, 38, 47, 50, 57, 65, 67, 68, 70, 73 and 79 did not have suppression.

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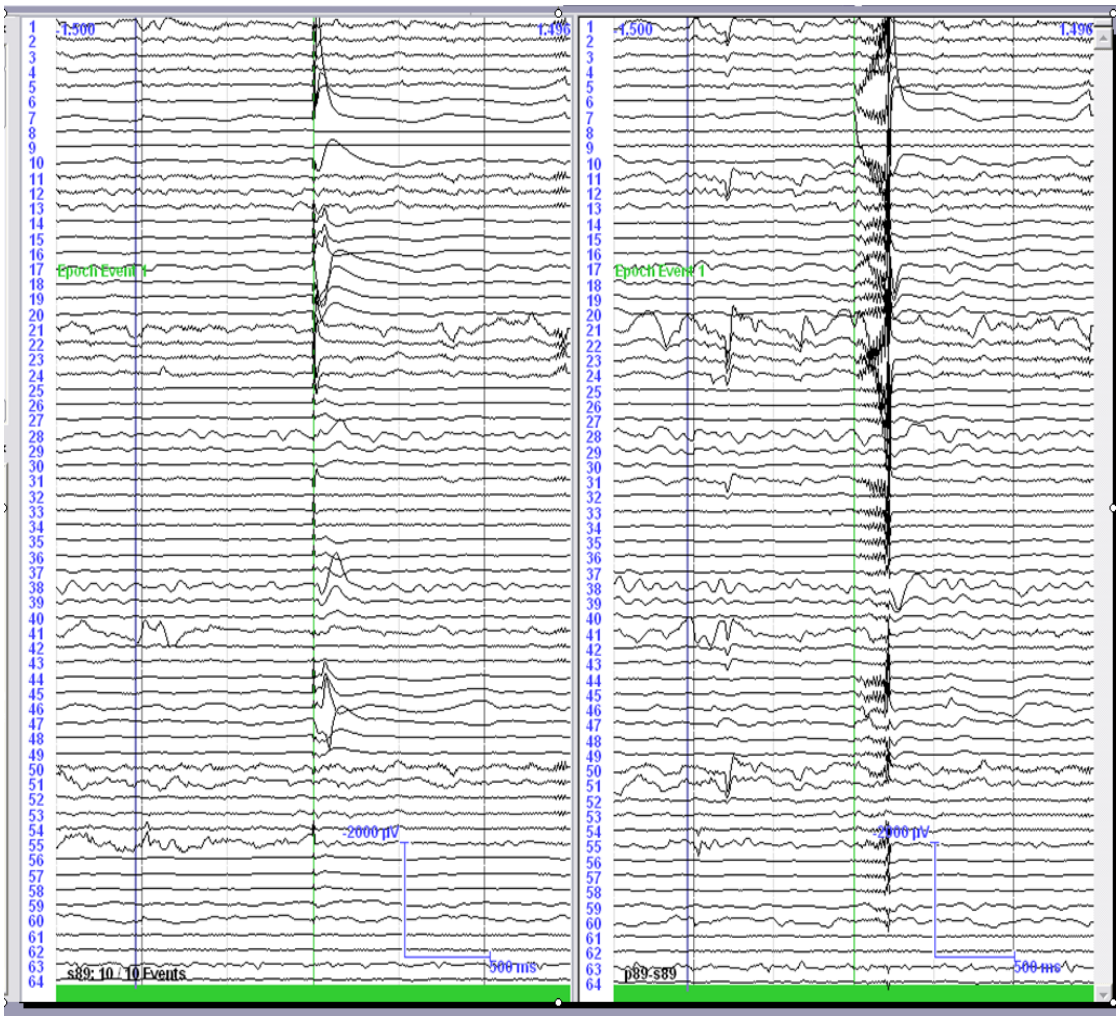

Figure 1. Patient 1. Suppression is seen in channels 14, 44, and 45. In this and the next figures of this appendix SPES is on the left and PPES is on the right. The stimulating electrodes and the polarity are shown on the bottom left part of each panel (in this case s89 for the SPES and p89-s89 for the PPES).

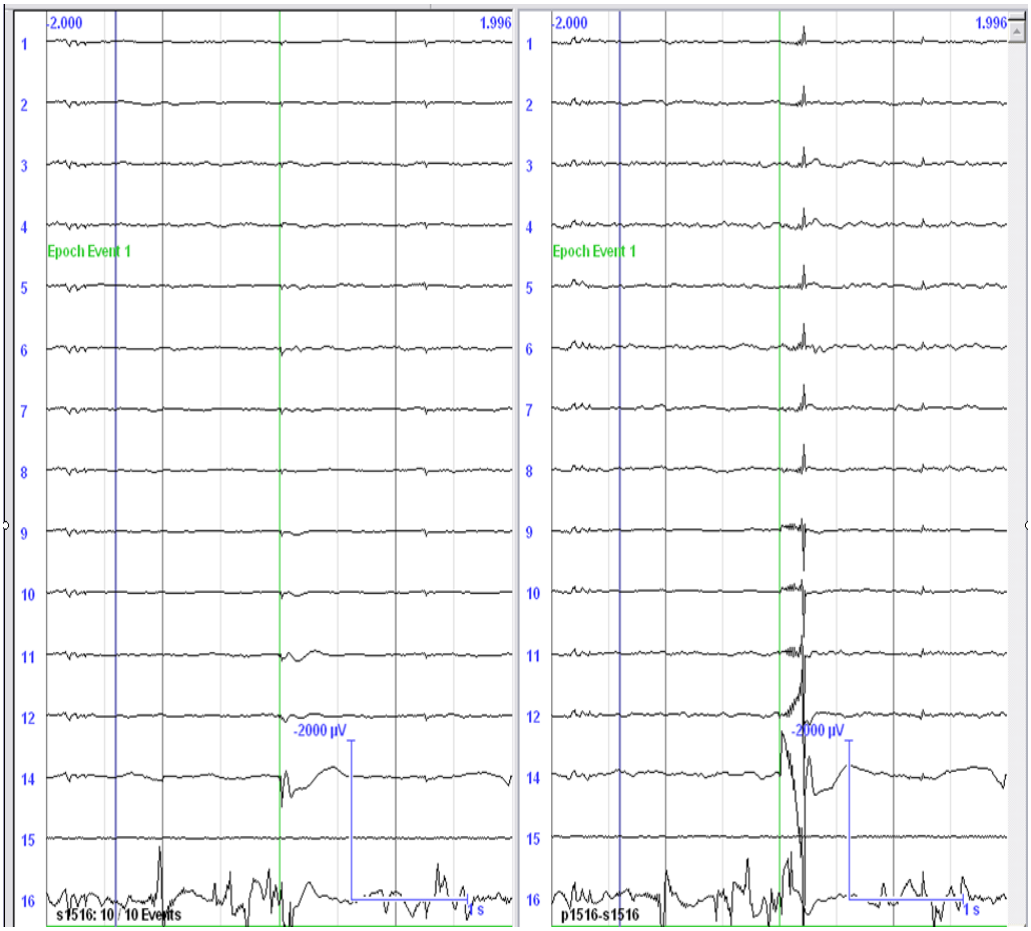

Figure 2. Patient 2. Suppression is seen in channels 10 and 11.

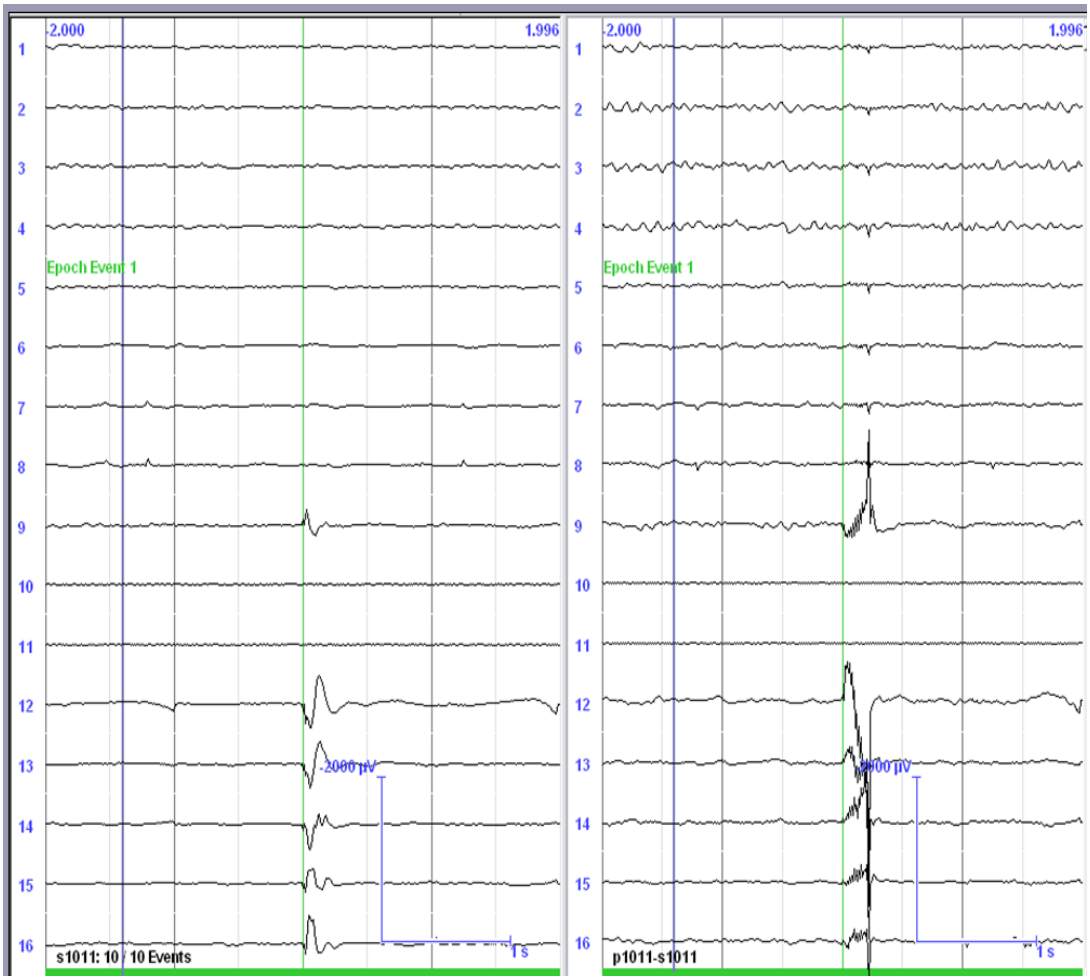

Figure 3. Patient 3. Suppression is seen in channels 14 and 15.

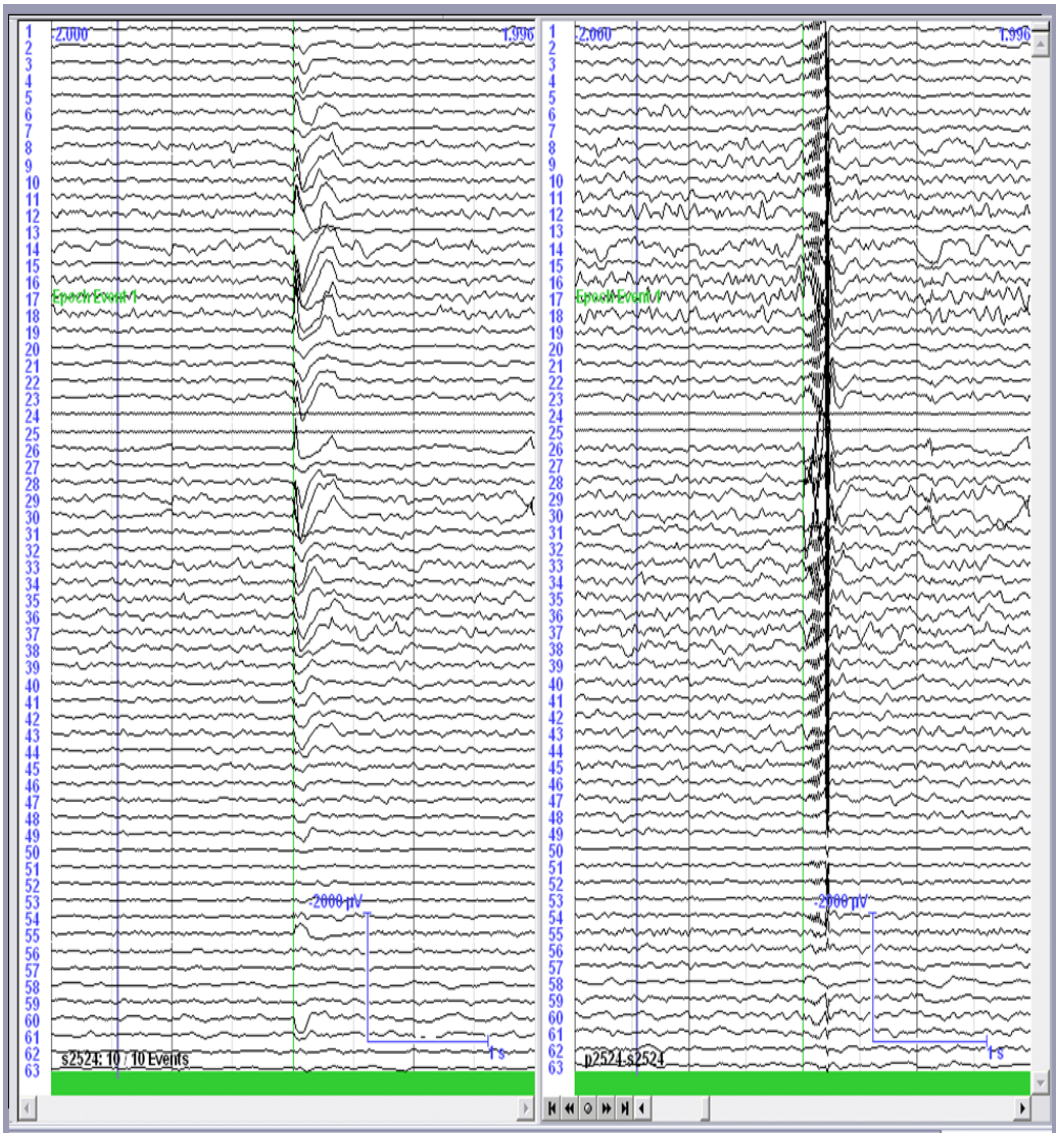

Figure 4. Patient 4. Suppression is seen in channels 2-4, 6, 9, 10, 13, 20, 21, 27, 36, 42, 43, 49 and 60. Inconclusive SO patient.

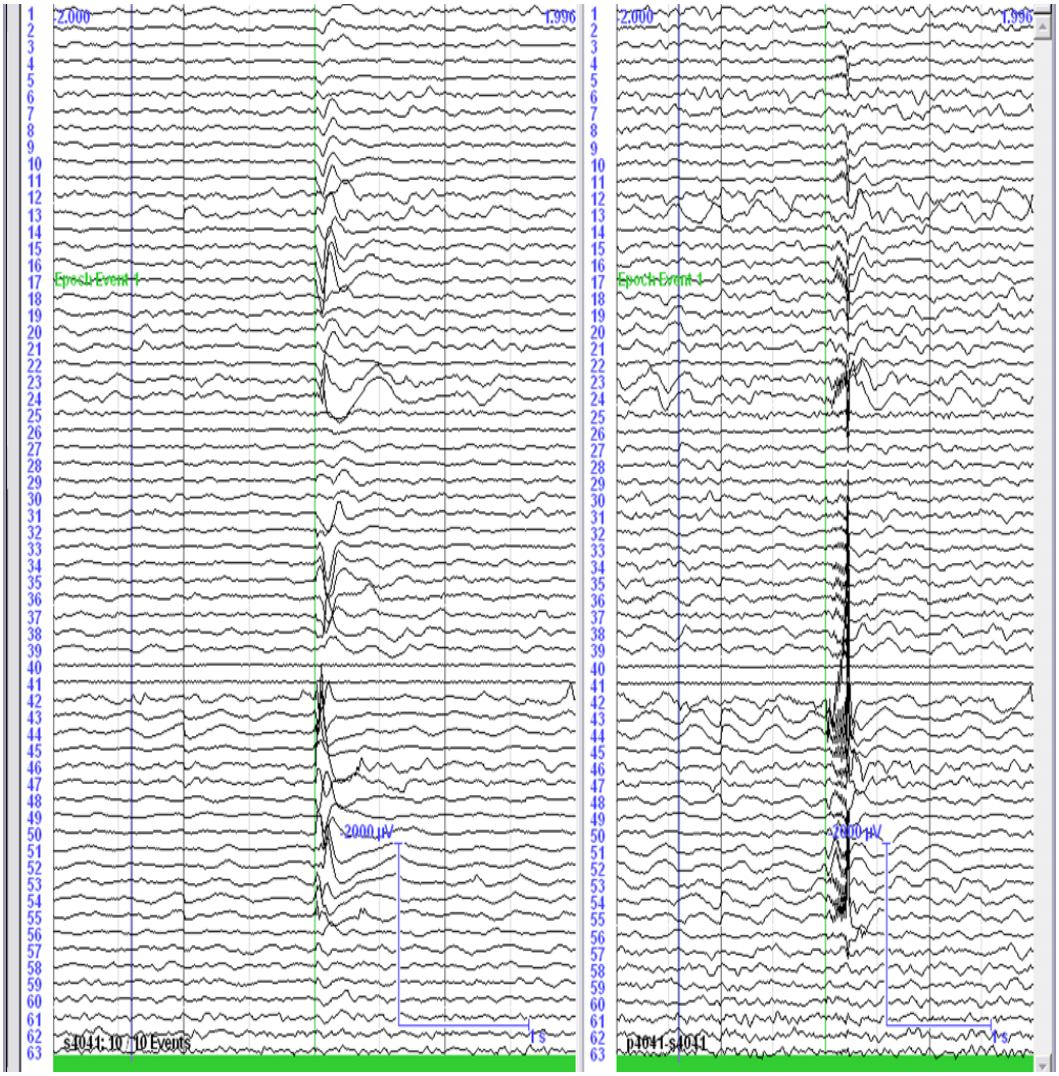

Figure 5. Patient 5. Suppression is seen in channels 29, 30, 31 and 34-37.

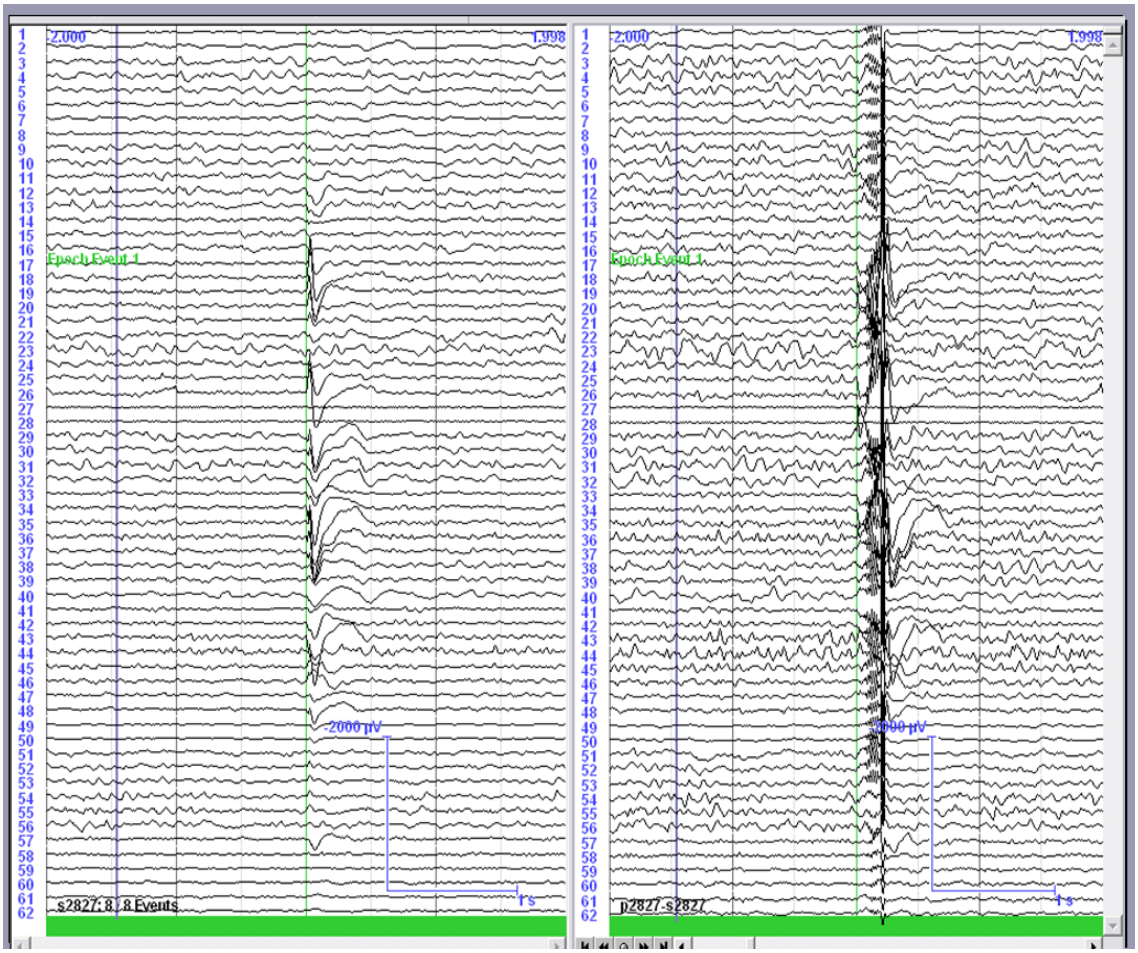

Figure 6. Patient 6. Suppression is seen in channels 31, 32, 38 and 40.

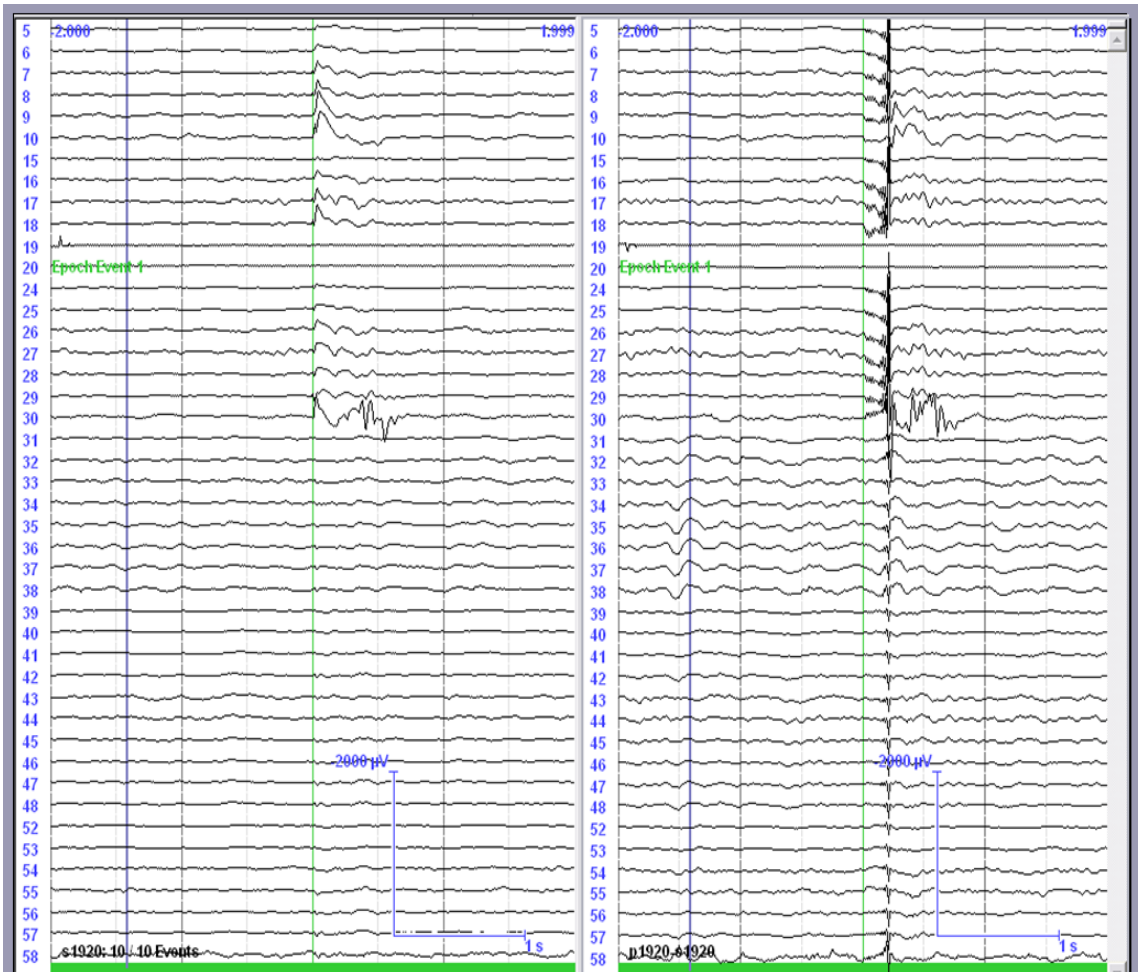

Figure 7. Patient 7. Suppression is seen in channels 17 and 18.

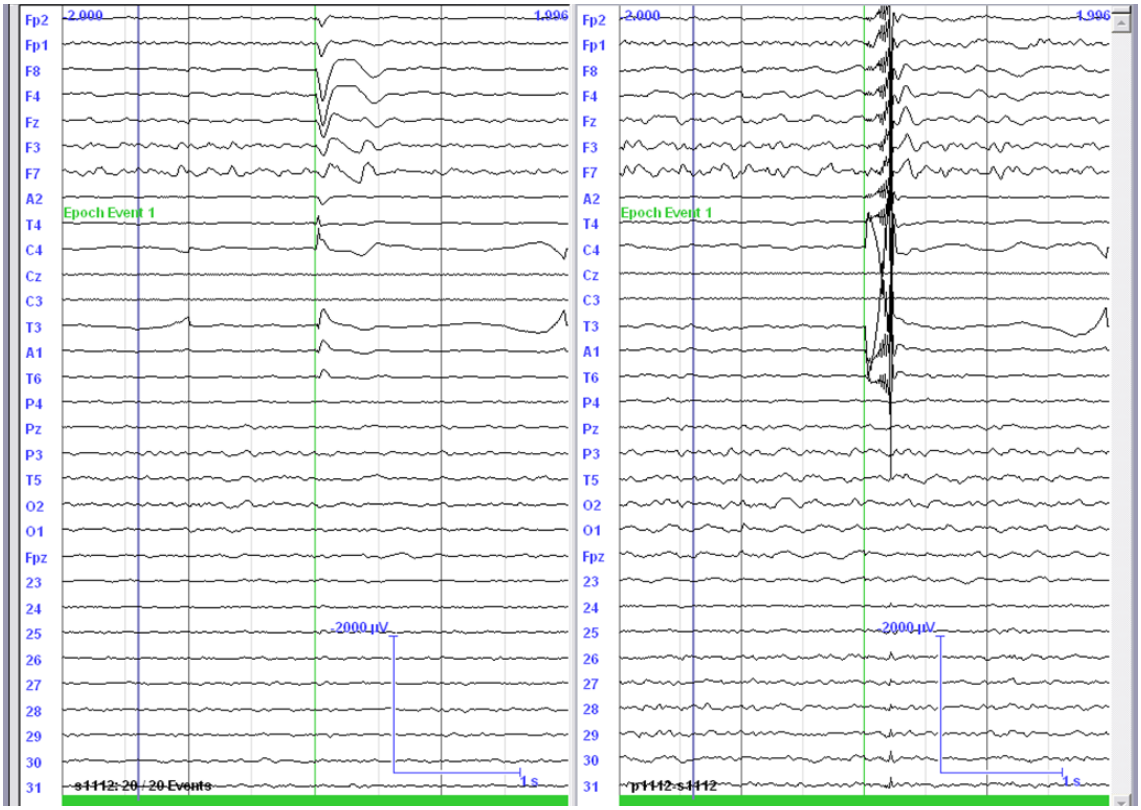

Figure 8. Patient 9. Suppression is seen in channels 3 (F8) and 4 (F4).

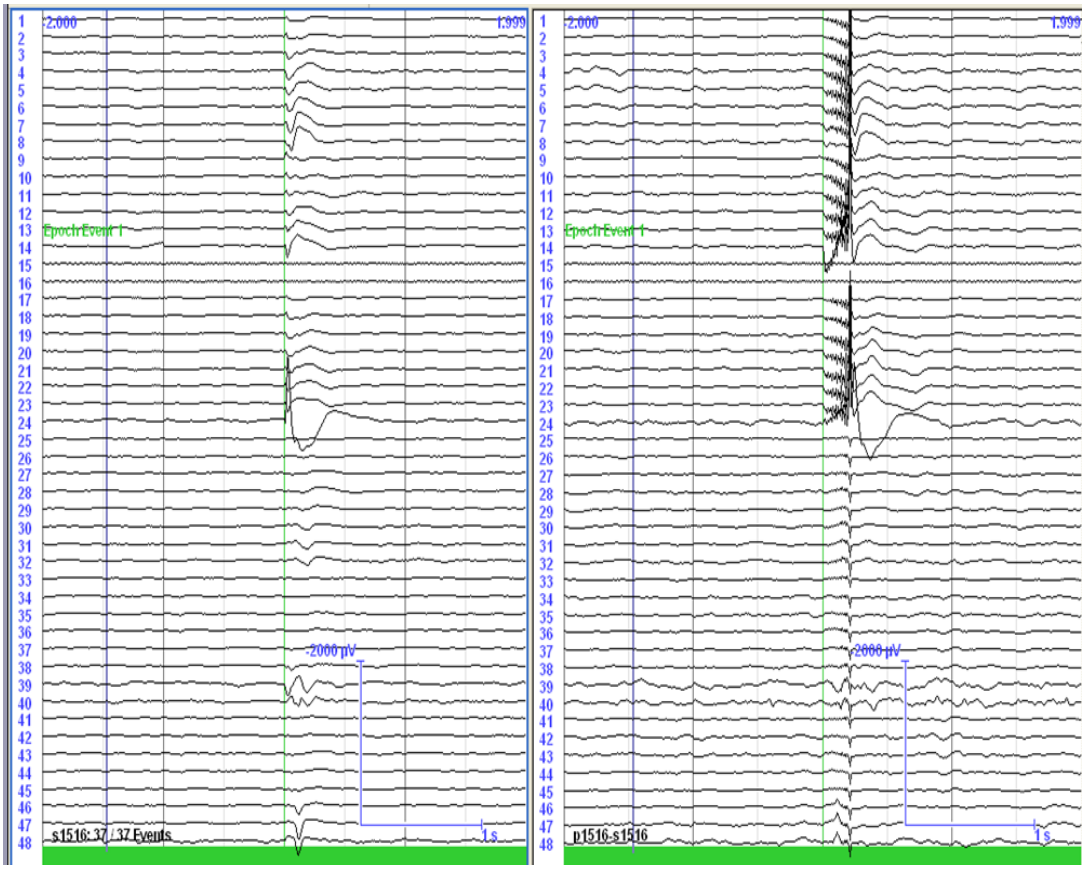

Figure 9. Patient 10. Suppression is seen in channels 30, 31, 46, 47 and 48.

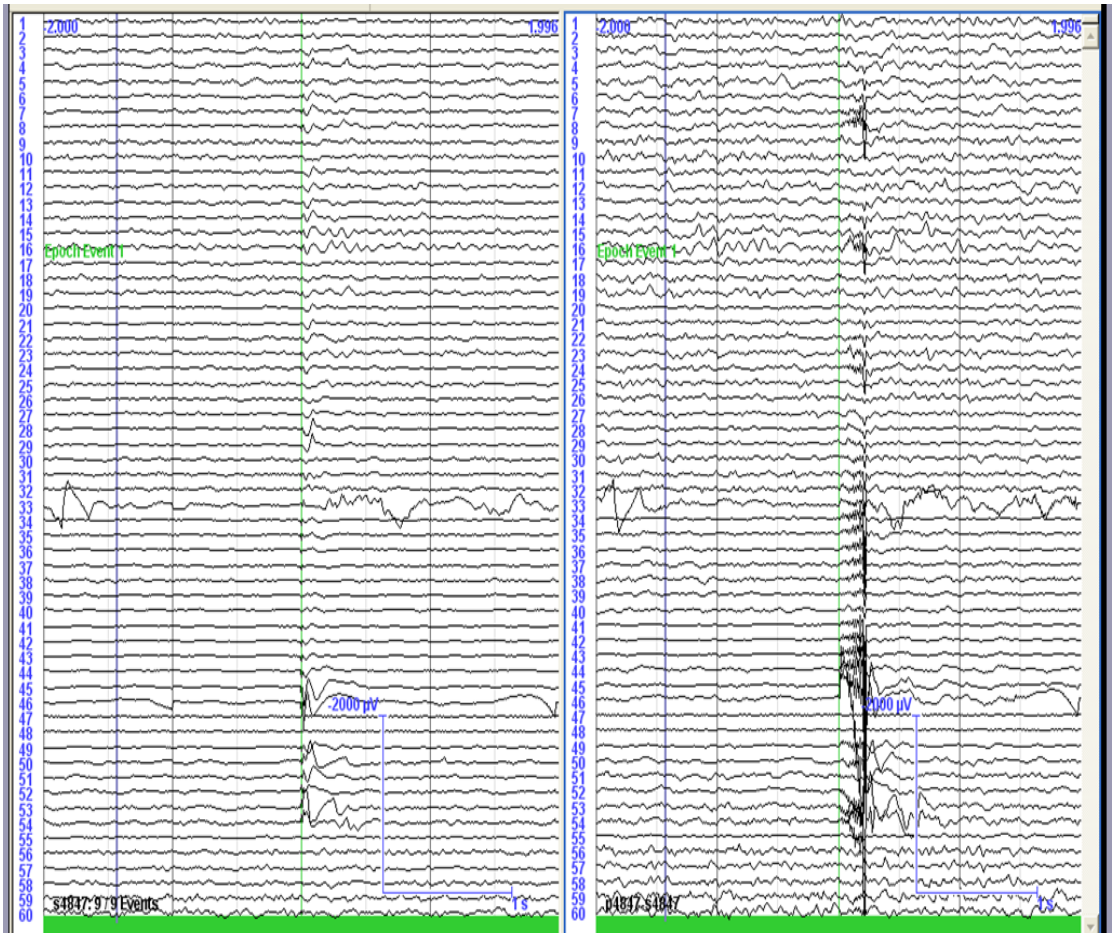

Figure 10. Patient 11. Suppression is seen in channels 28 and 29.

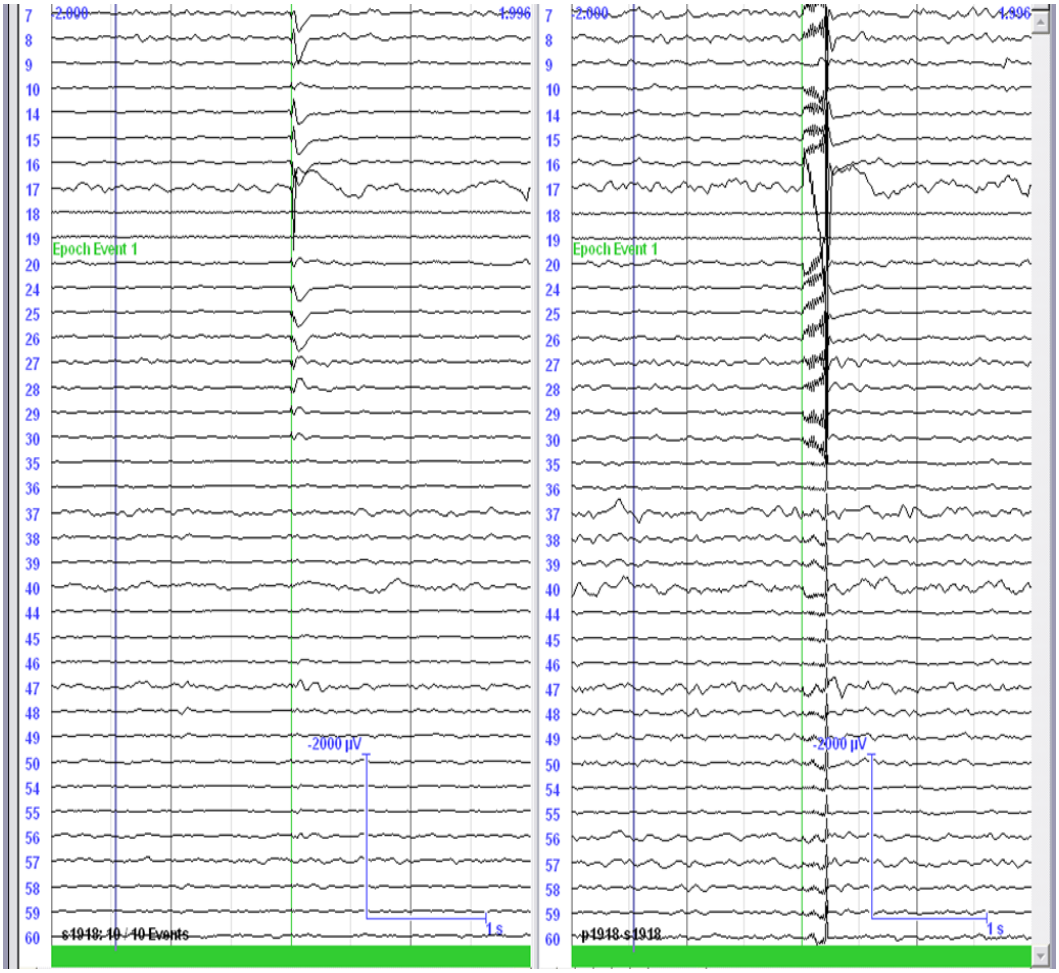

Figure 11. Patient 12. Suppression is seen in channel 26.

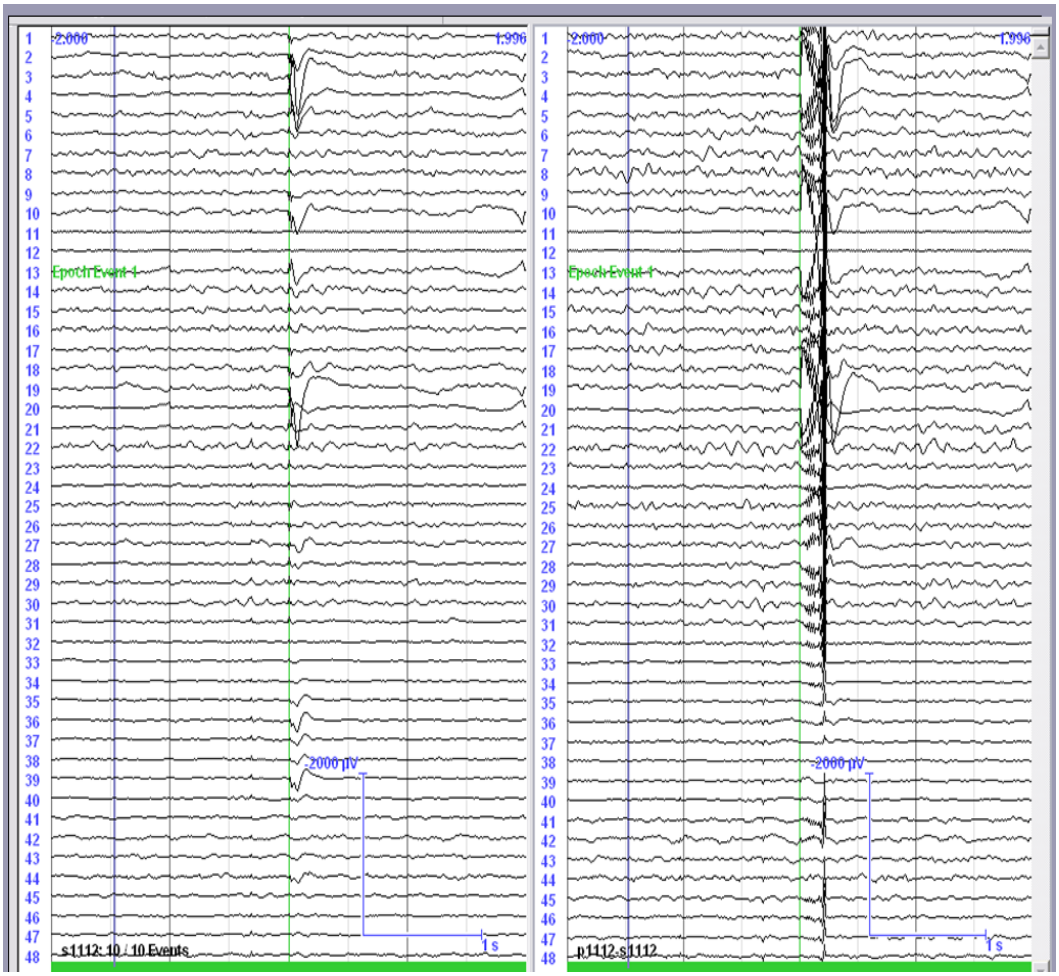

Figure 12. Patient 13. Suppression is seen in channels 35-39 and 44.

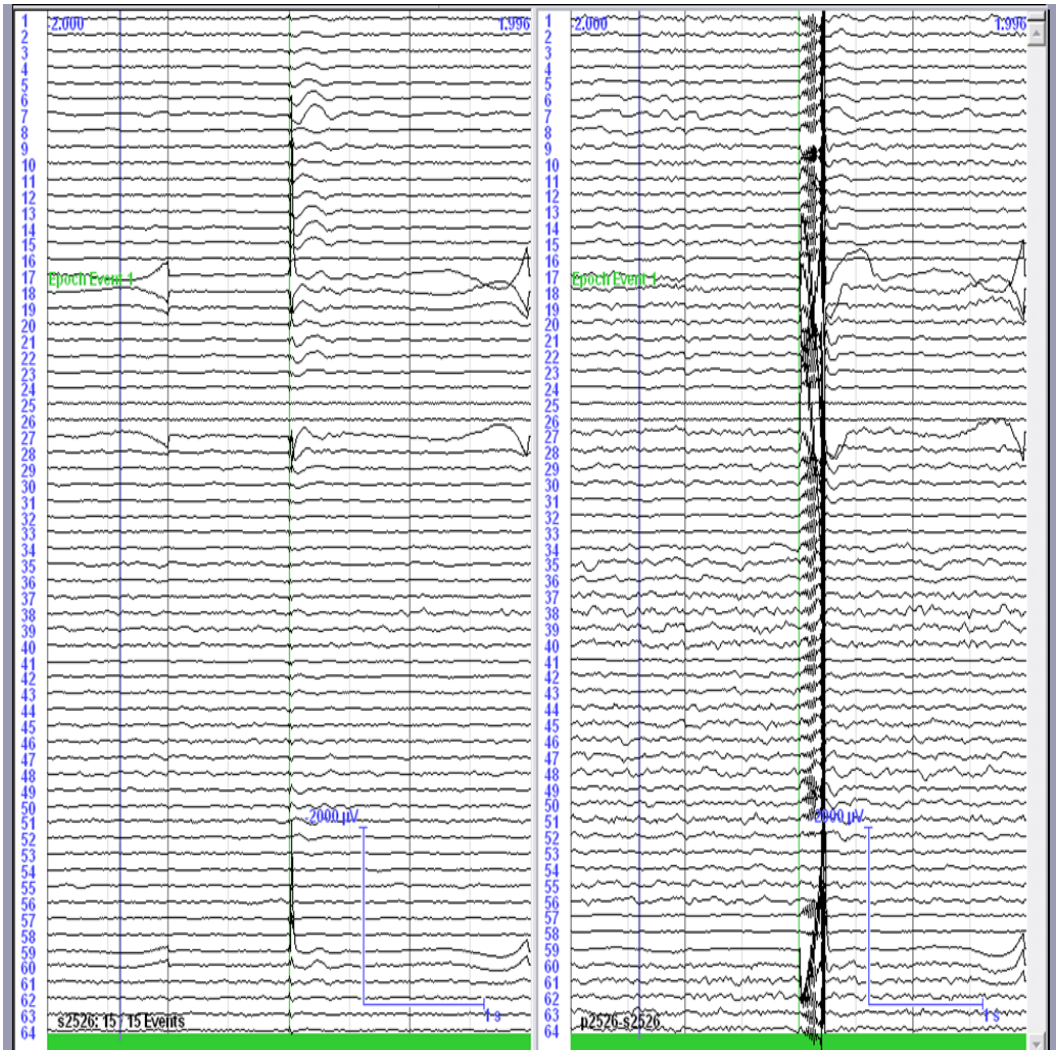

Figure 13. Patient 14. Suppression is seen in channels 13-15.

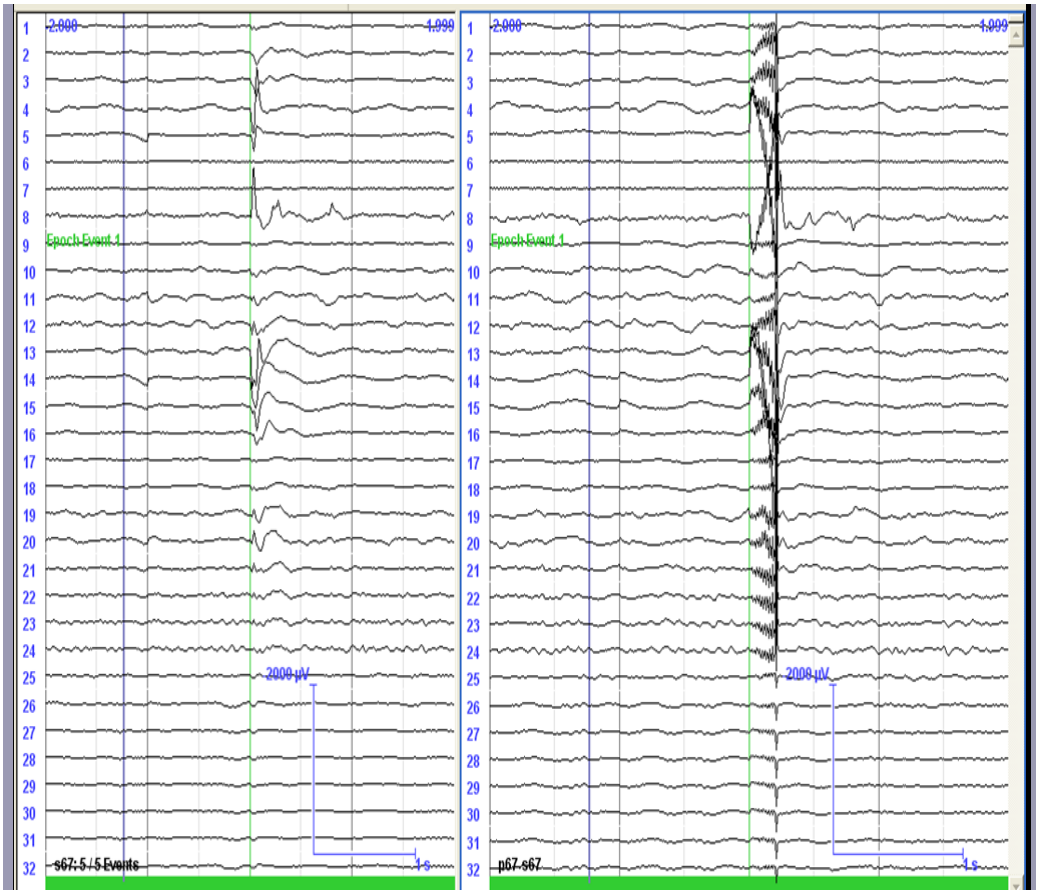

Figure 14. Patient 15. Suppression is seen in channel 19.

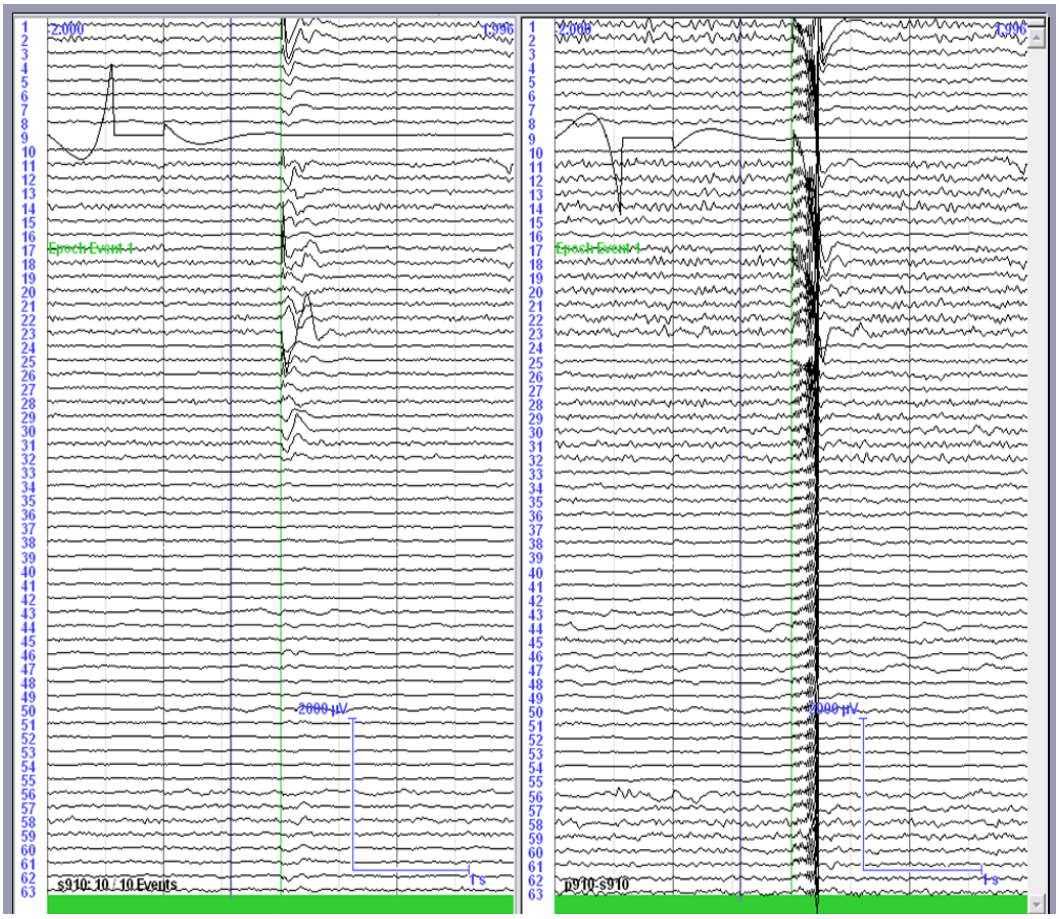

Figure 15. Patient 16. Suppression is seen in channels 6, 7, 30 and 31.

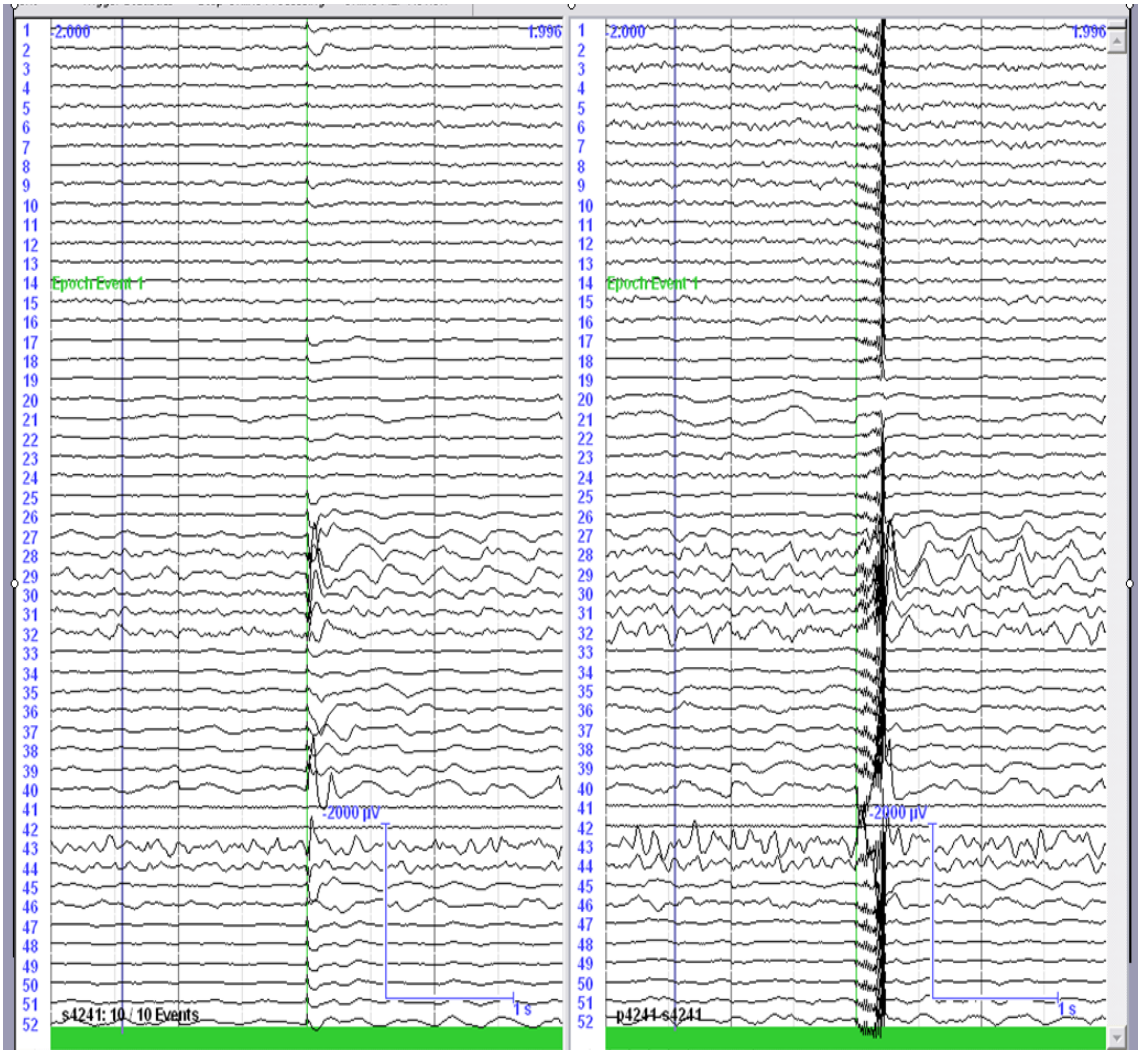

Figure 16. Patient 17. Suppression is seen in channel 2.

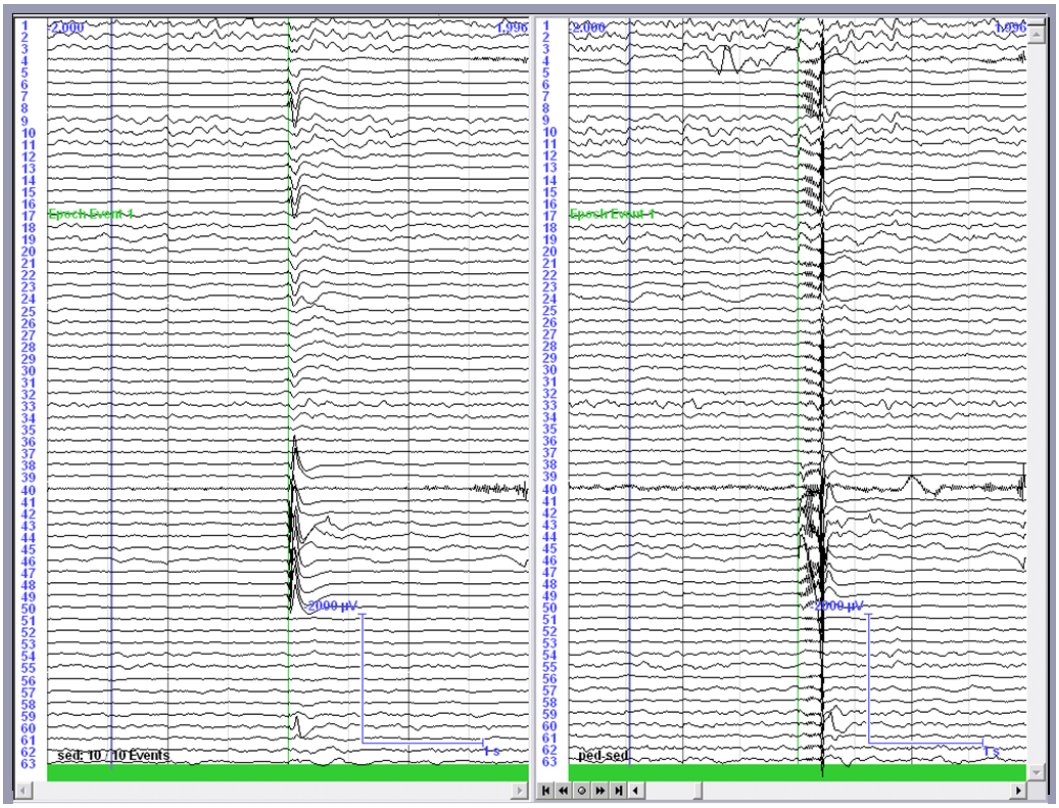

Figure 17. Patient 18. Suppression is seen in channels 5 and 13. In this case the stimulating electrodes are extra electrodes.

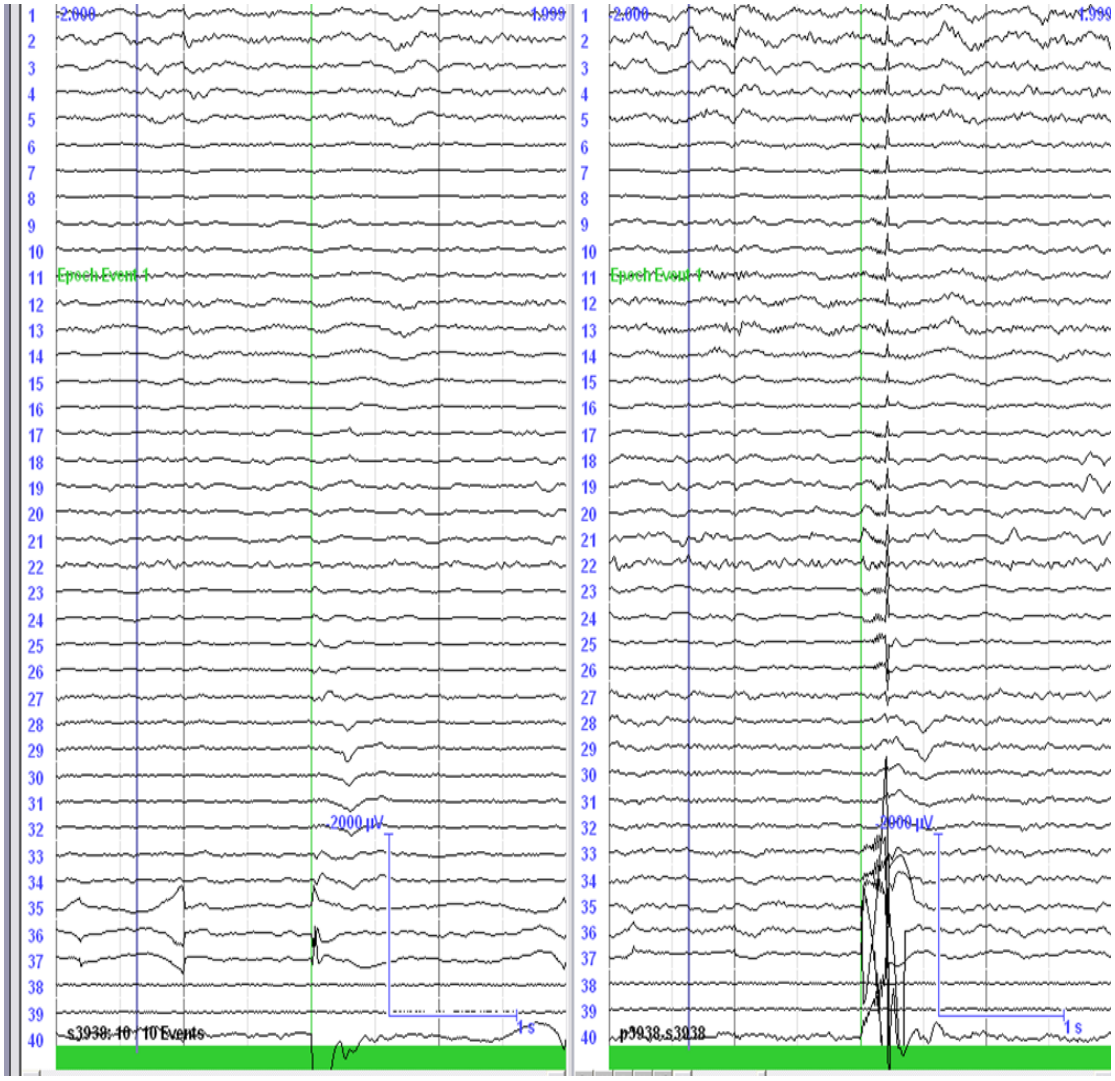

Figure 18. Patient 19. Suppression is seen in channel 27.

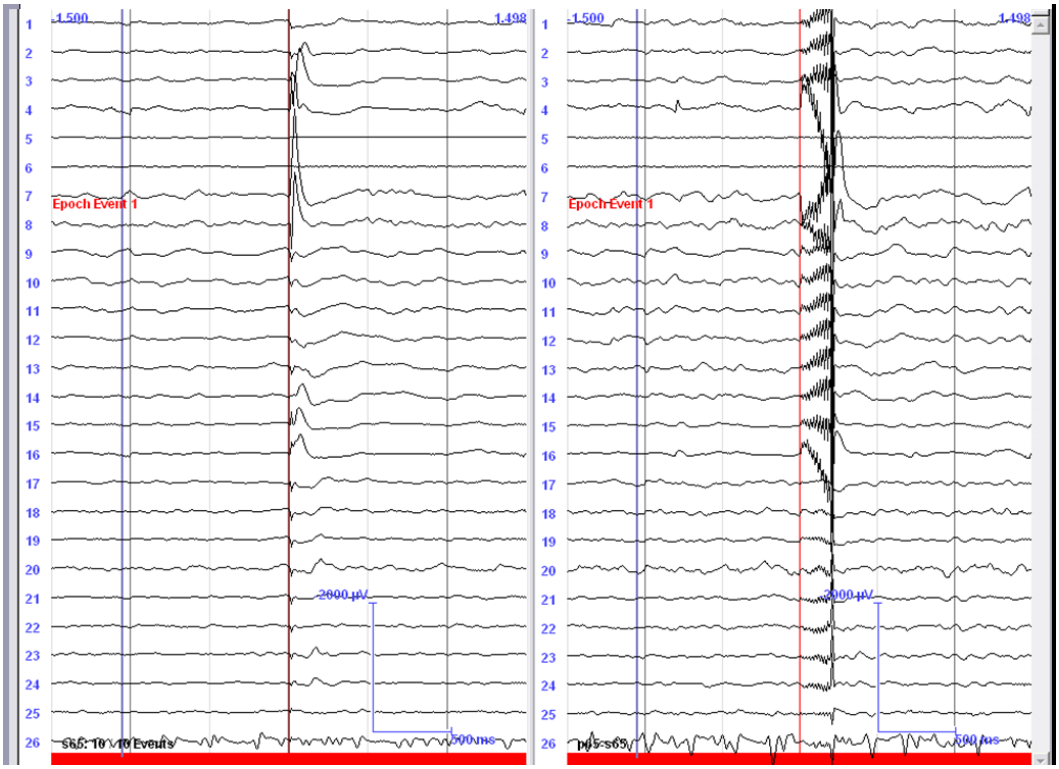

Figure 19. Patient 21. Suppression is seen in channel 15.

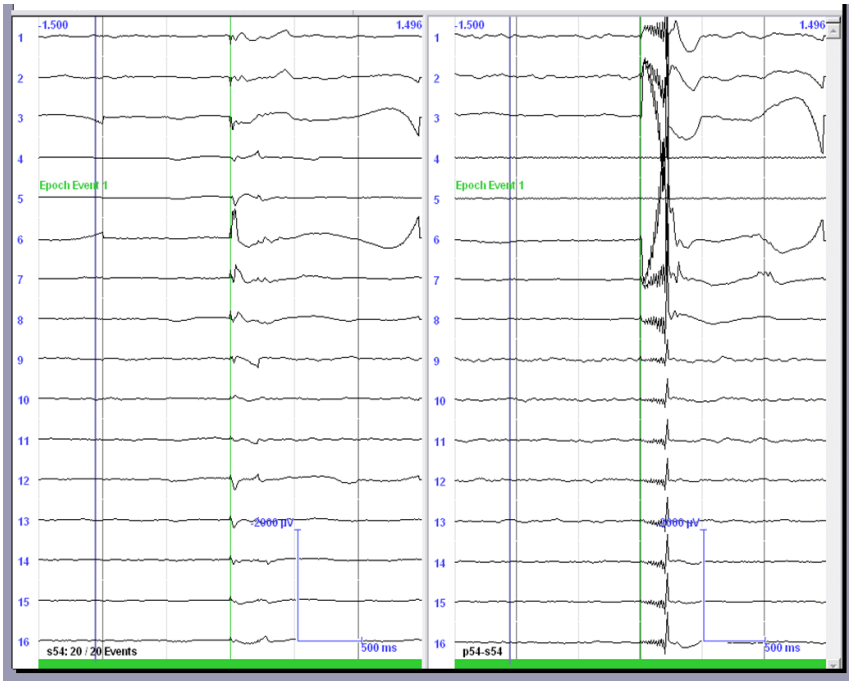

Figure 20. Patient 23. Suppression is seen in channels 9, 12 and 13.

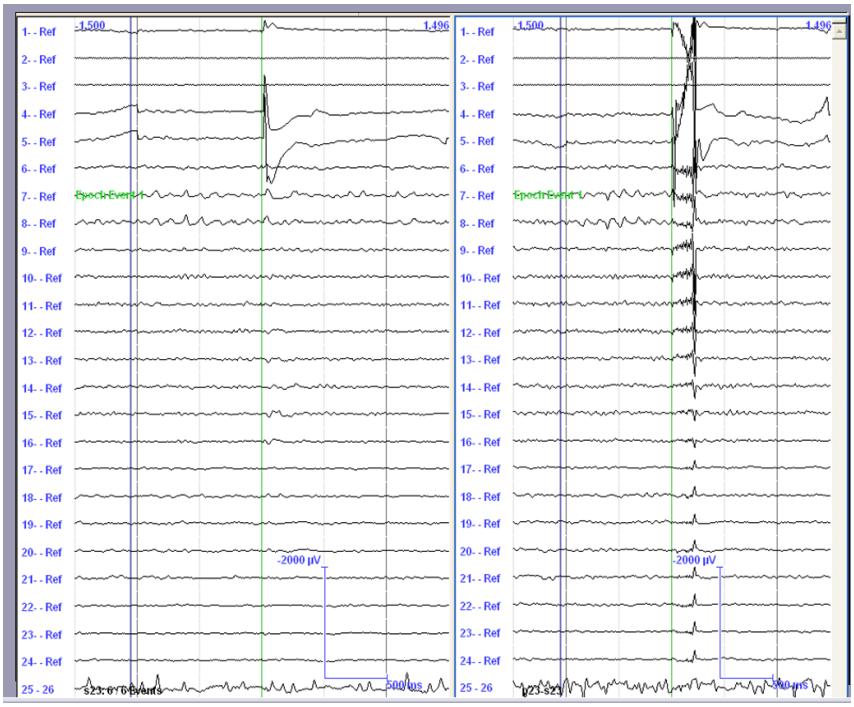

Figure 21. Patient 24. Suppression is seen in channel 16. Inconclusive SO patient.

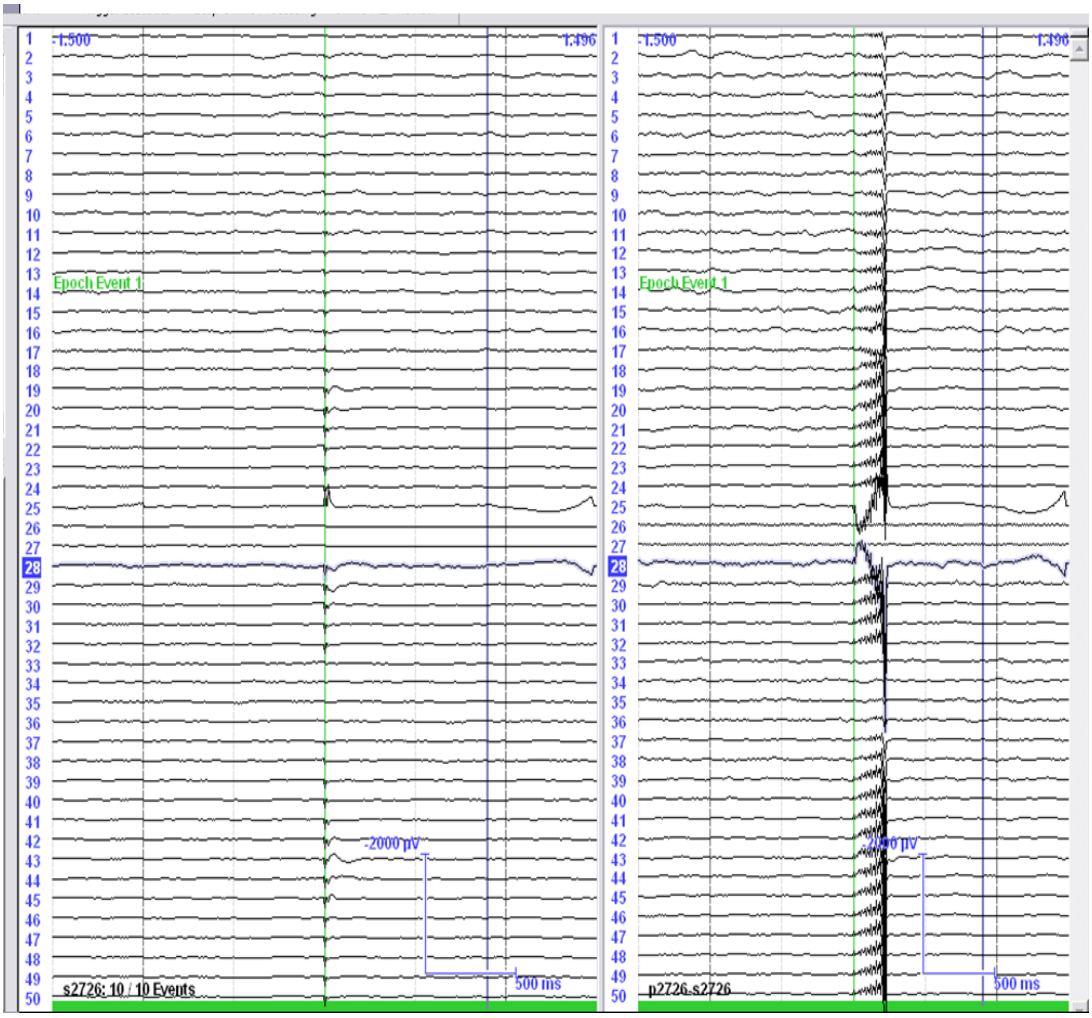

Figure 22. Patient 25. Suppression is seen in channels 19, 43 and 44.

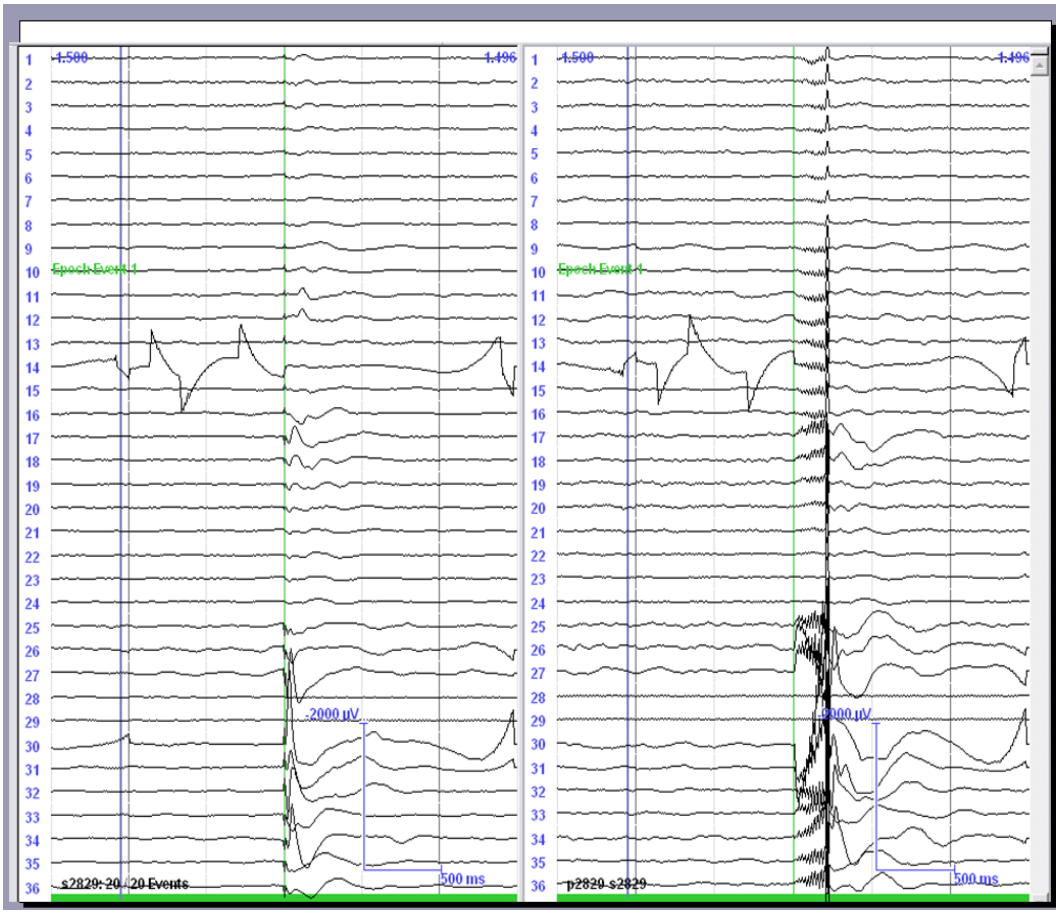

Figure 23. Patient 26. Suppression is seen in channels 1, 10, 11 and 19.

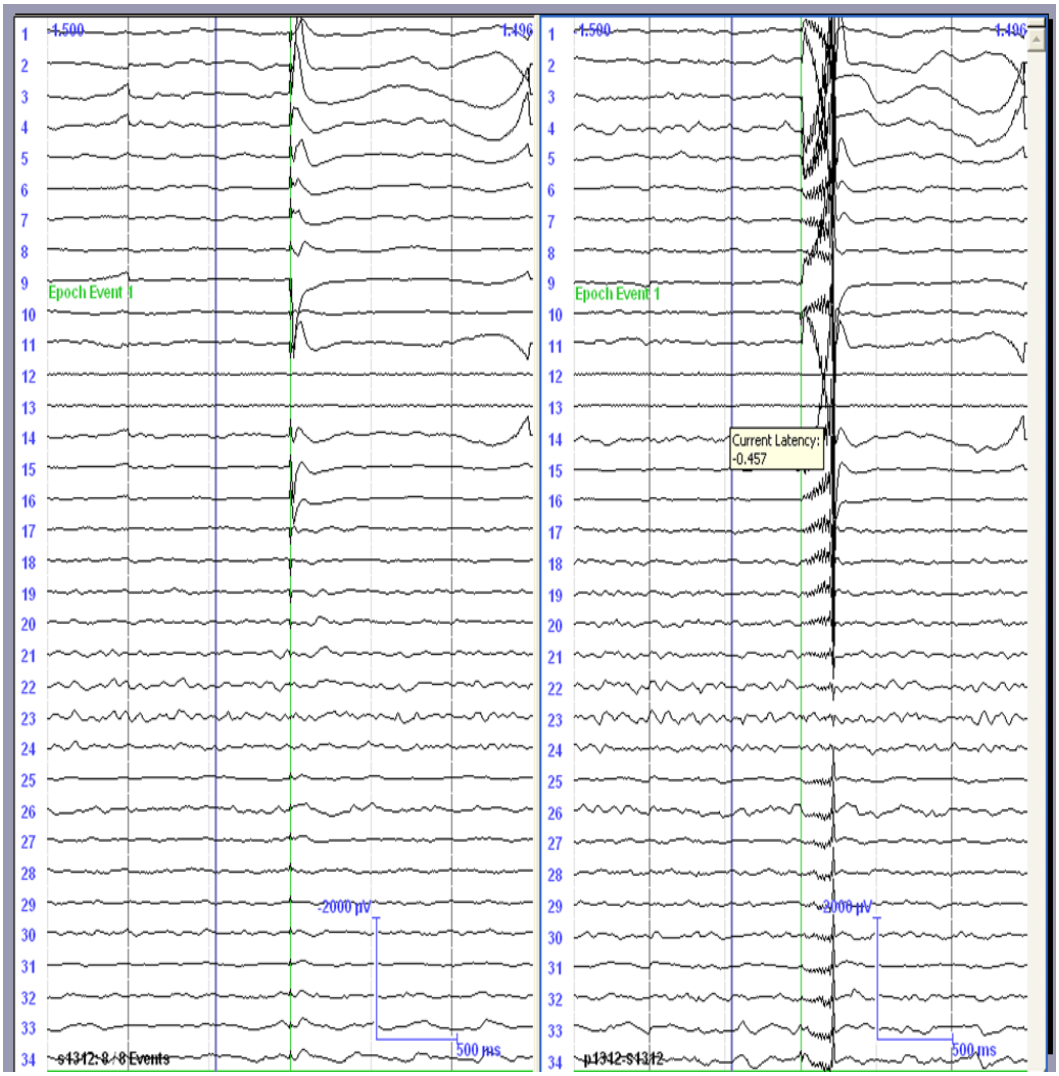

Figure 24. Patient 22. Suppression is seen in channels 8 and 16.

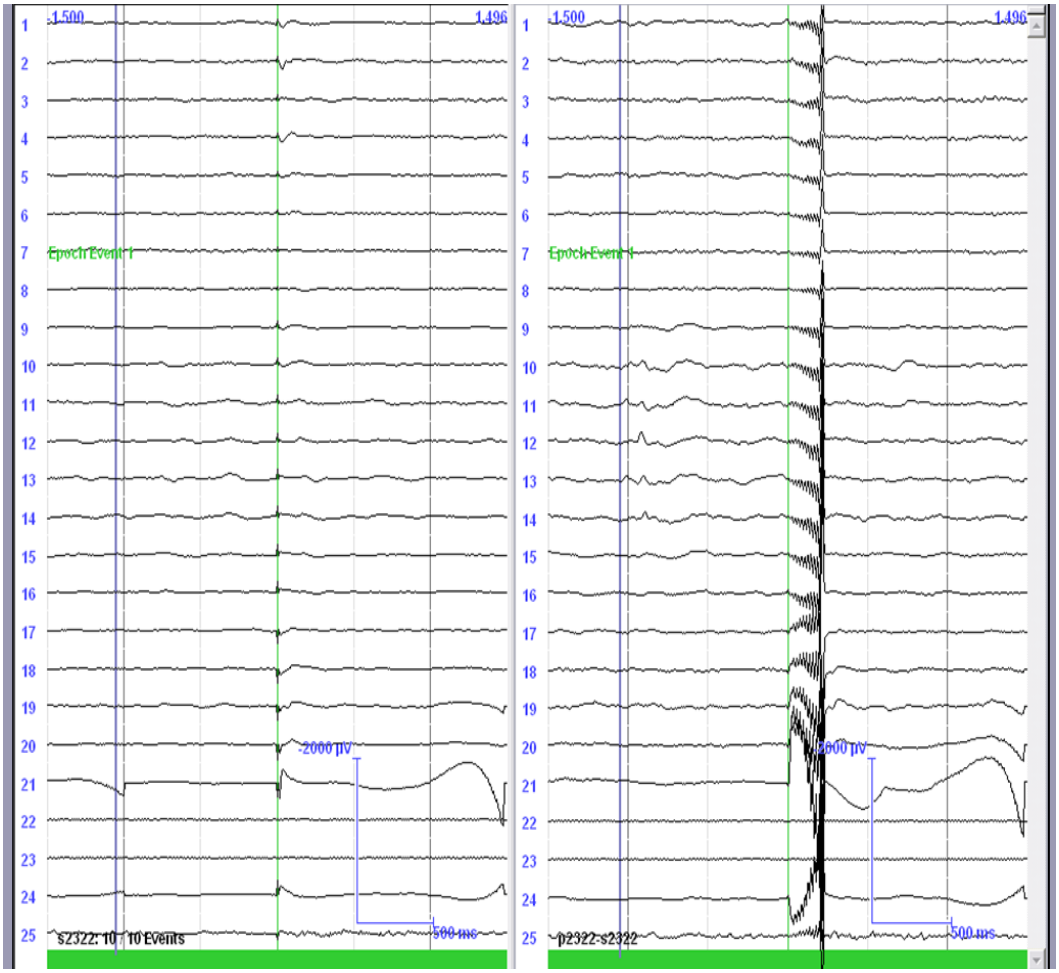

Figure 25. Patient 28. Suppression is seen in channels 1 and 4.

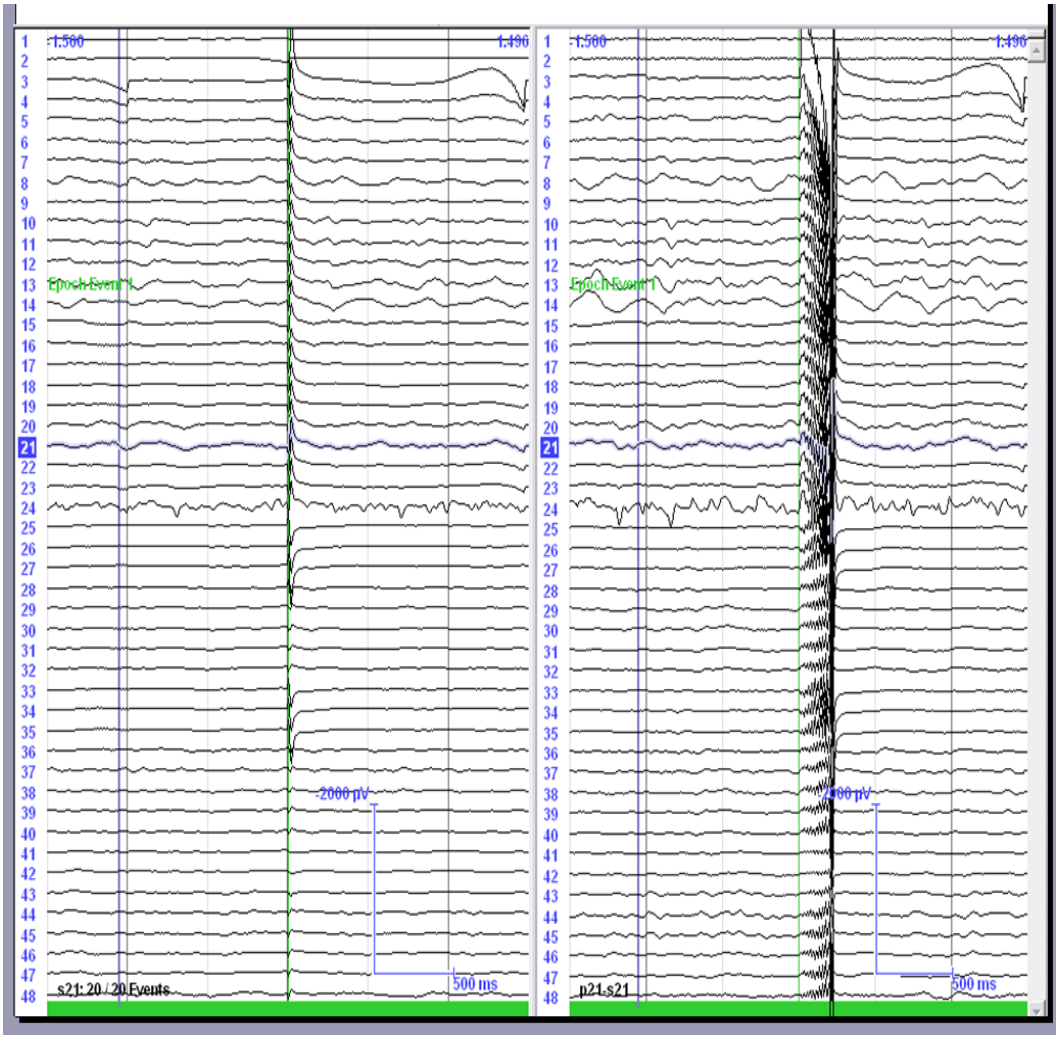

Figure 26. Patient 29. Suppression is seen in channels 6, 7, 9-13, 15 and 16.

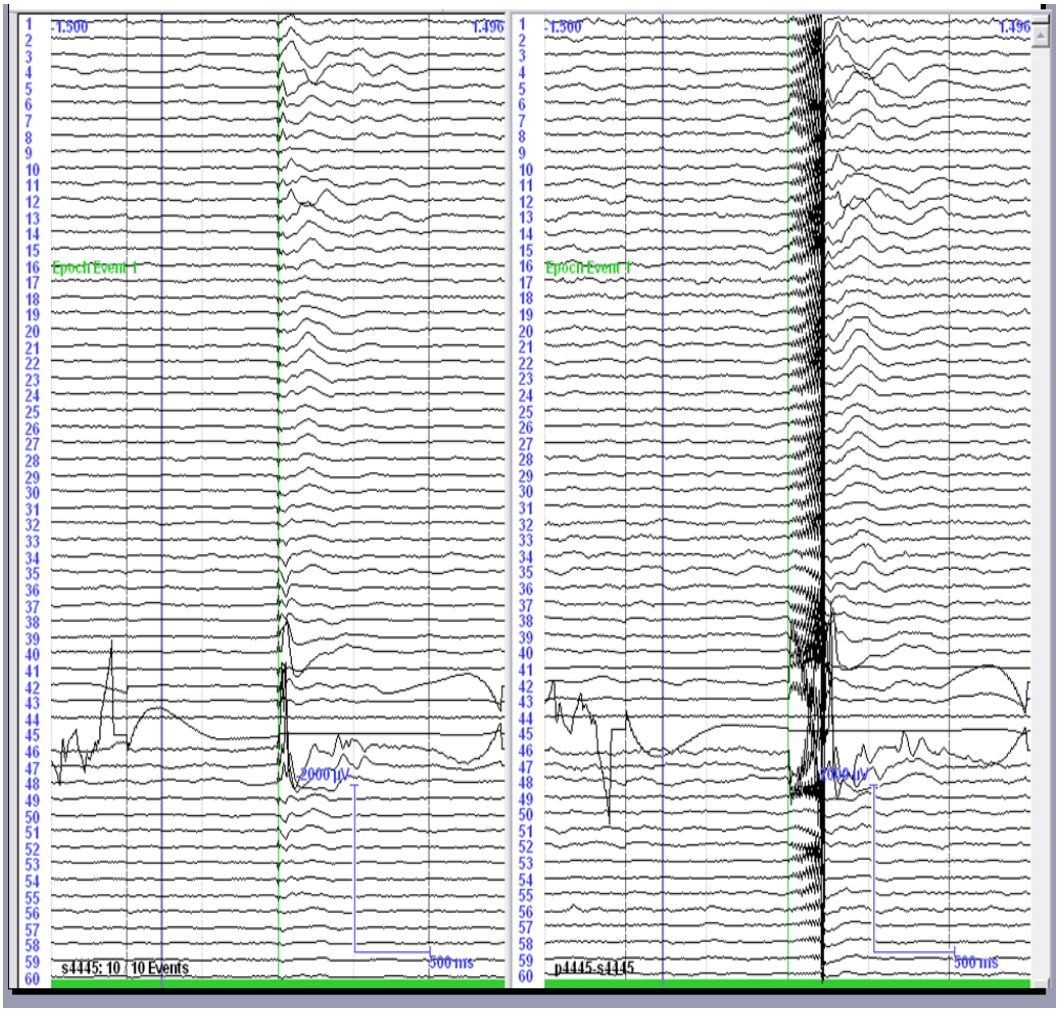

Figure 27. Patient 30. Suppression is seen in channel 49.

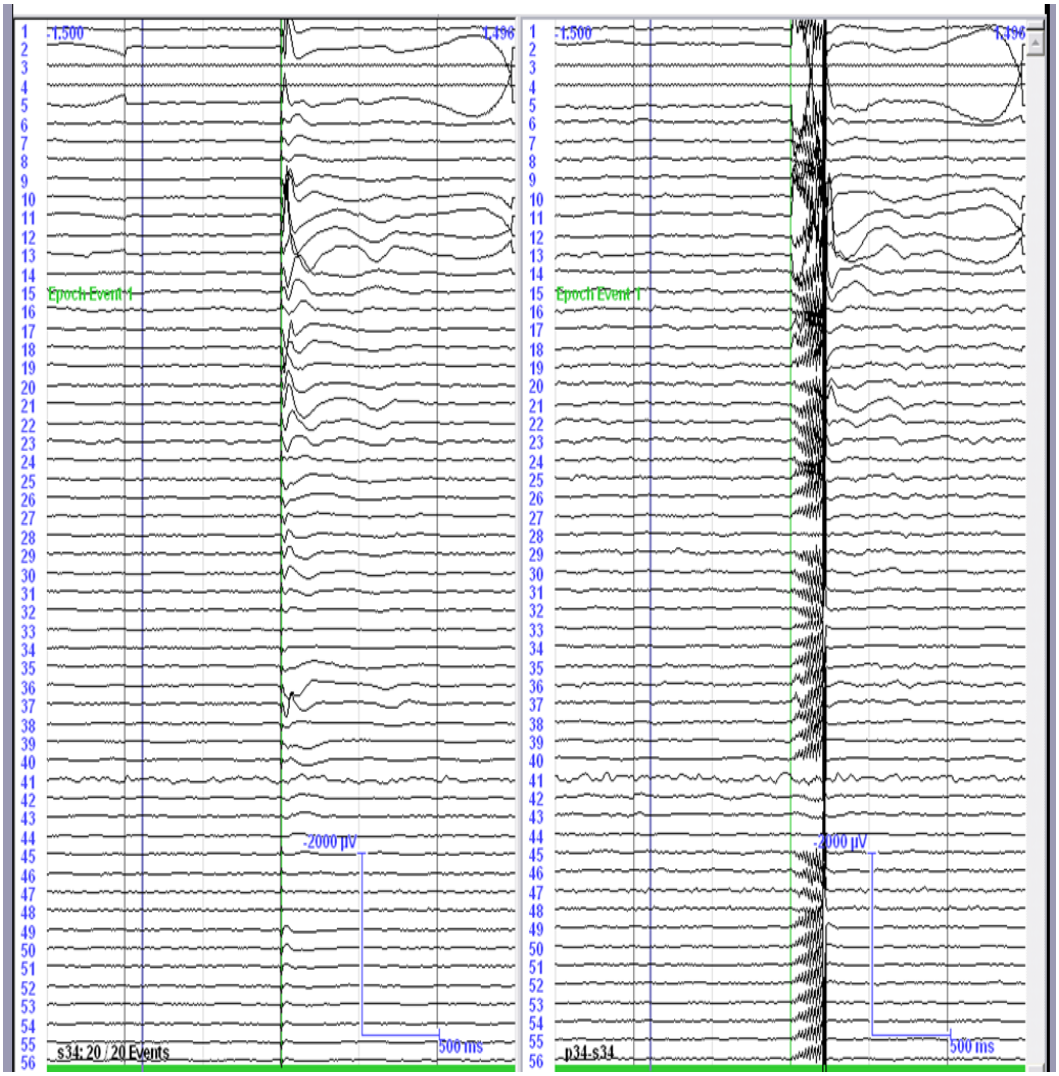

Figure 28. Patient 31. Suppression is seen in channels 16, 19, 22, 36, 37, 50-54.

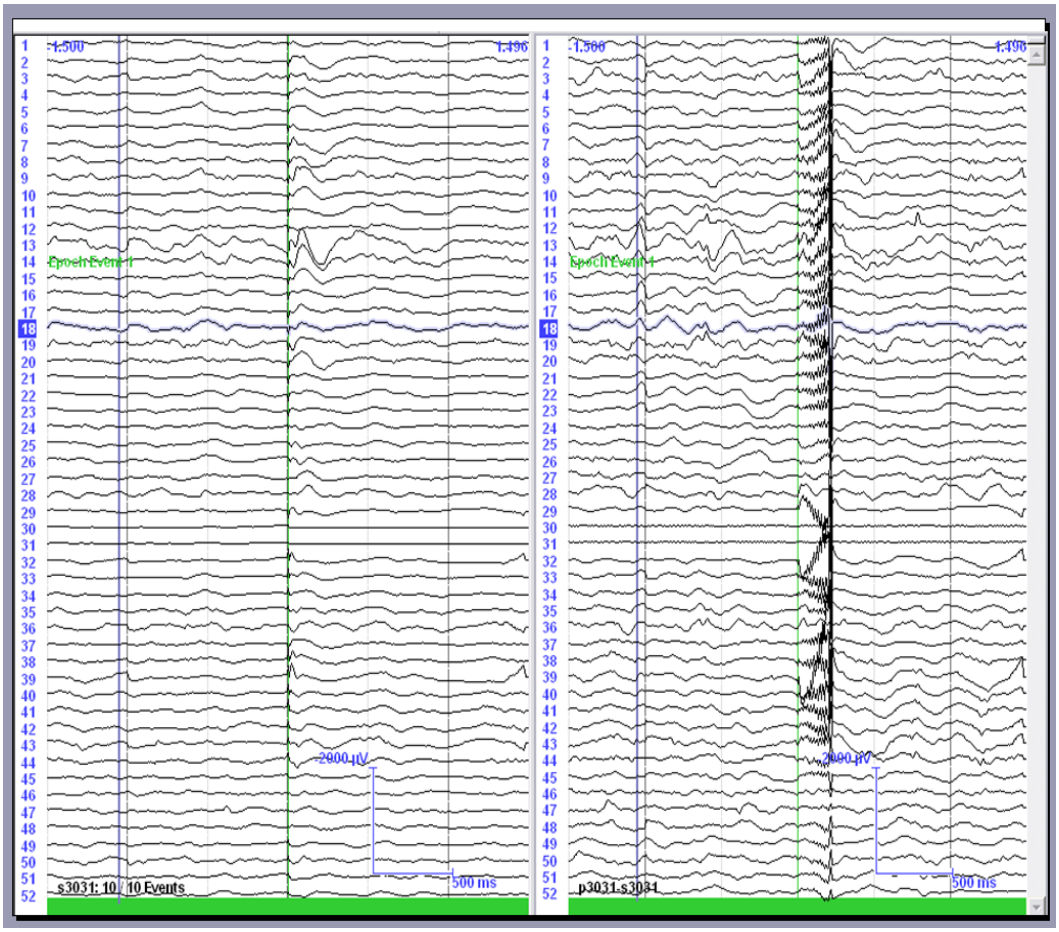

Figure 29. Patient 32. Suppression is seen in channels 10, 15, 17 and 28.

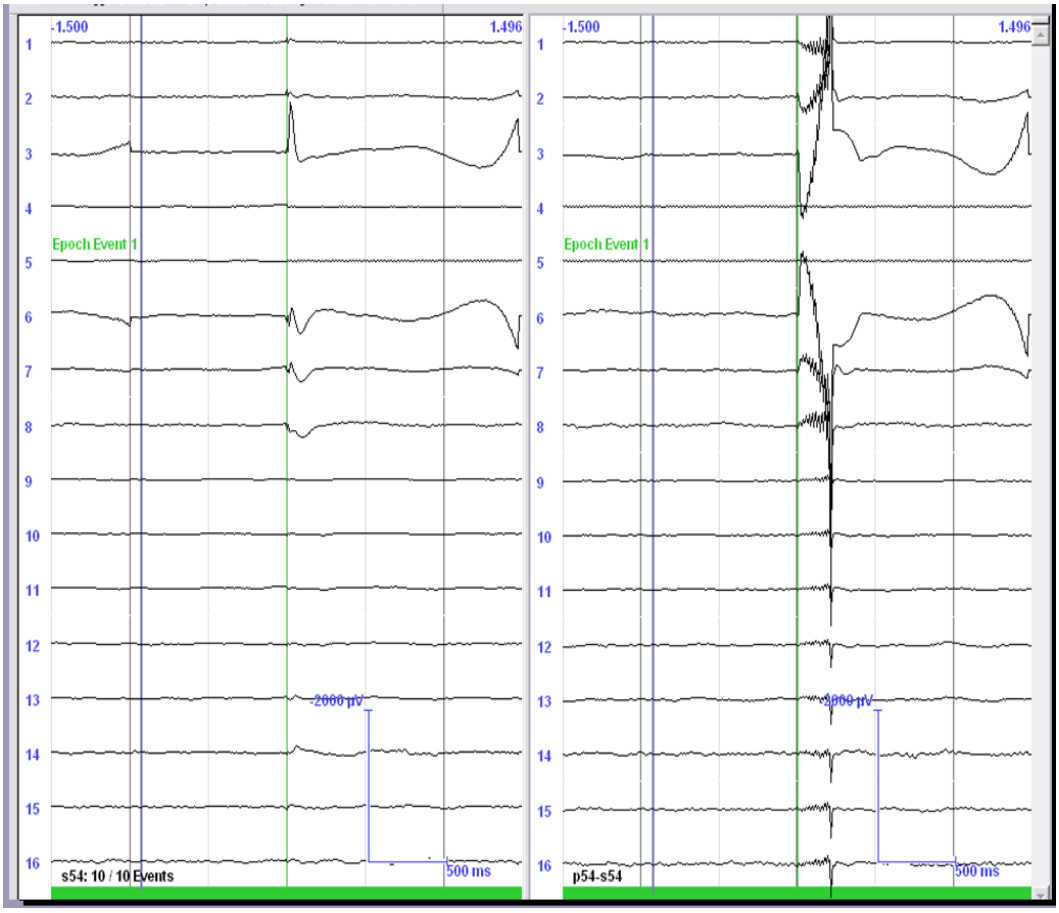

Figure 30. Patient 33. Suppression is seen in channels 8 and 14.

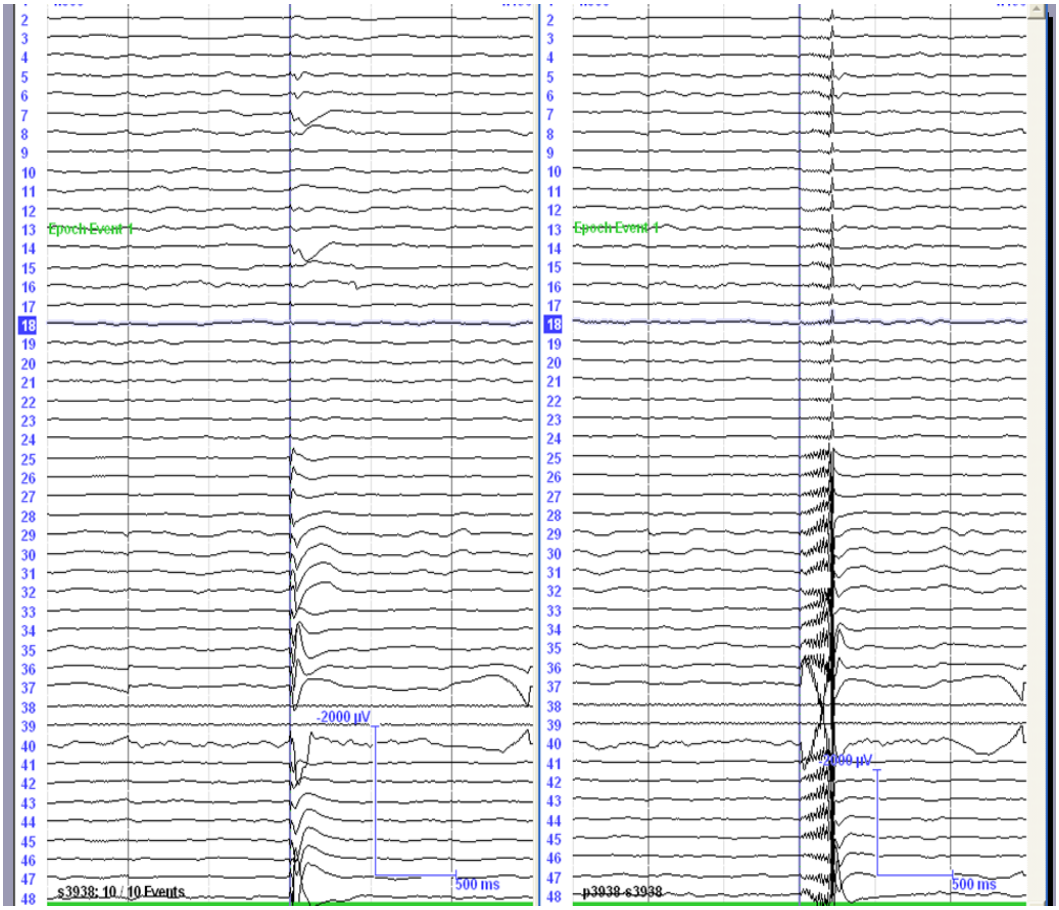

Figure 31. Patient 34. Suppression is seen in channels 7, 8 and 14.

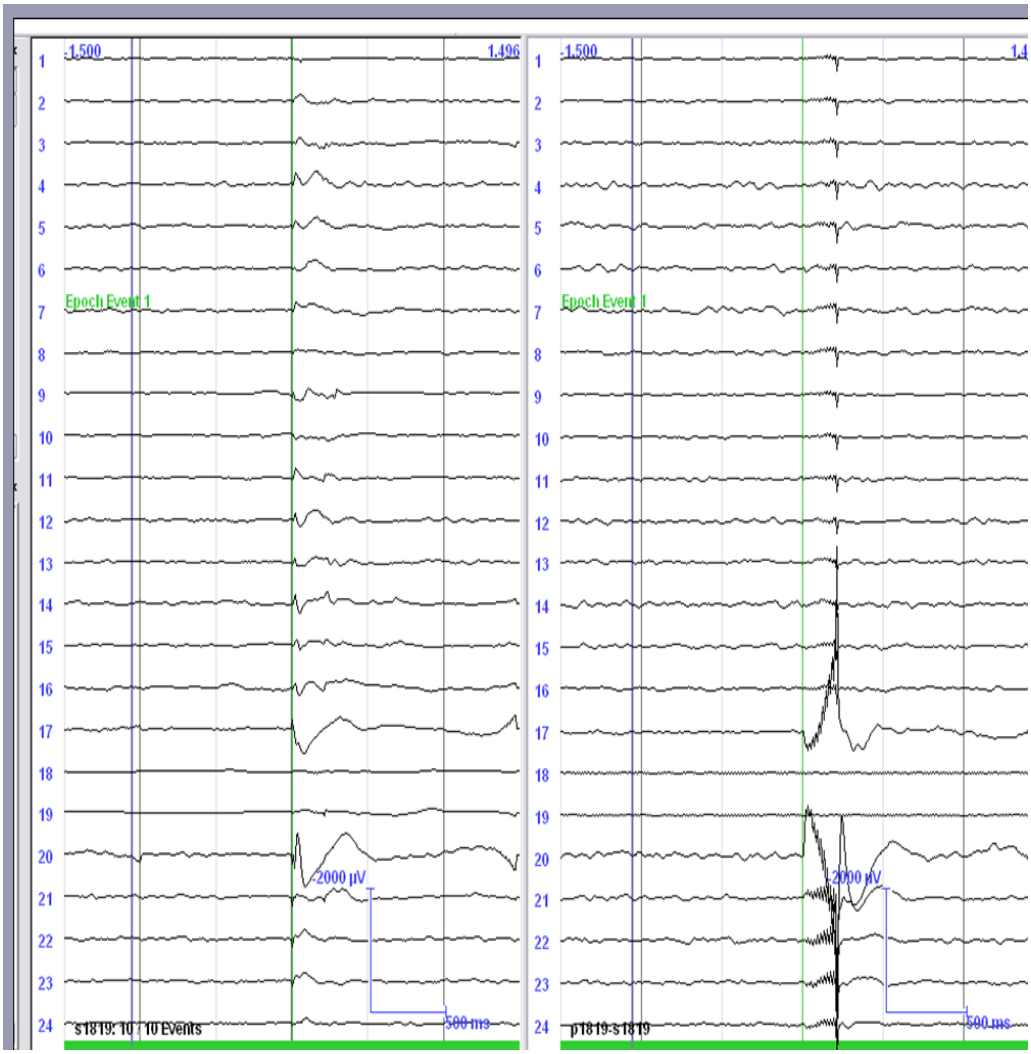

Figure 32. Patient 35. Suppression is seen in channels 4-7,9 and 11-16.

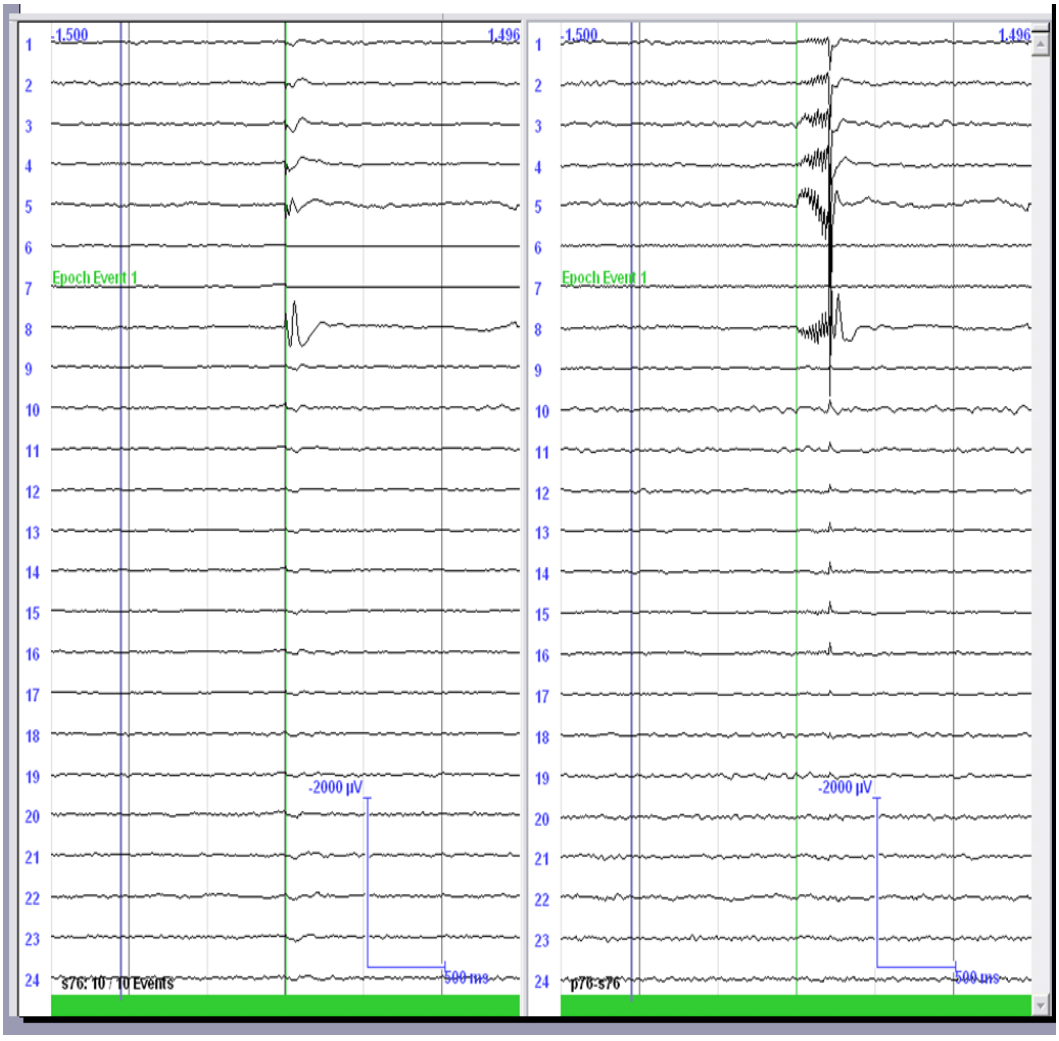

Figure 33. Patient 36. Suppression is seen in channels 9, 10, 16 and 21-24.

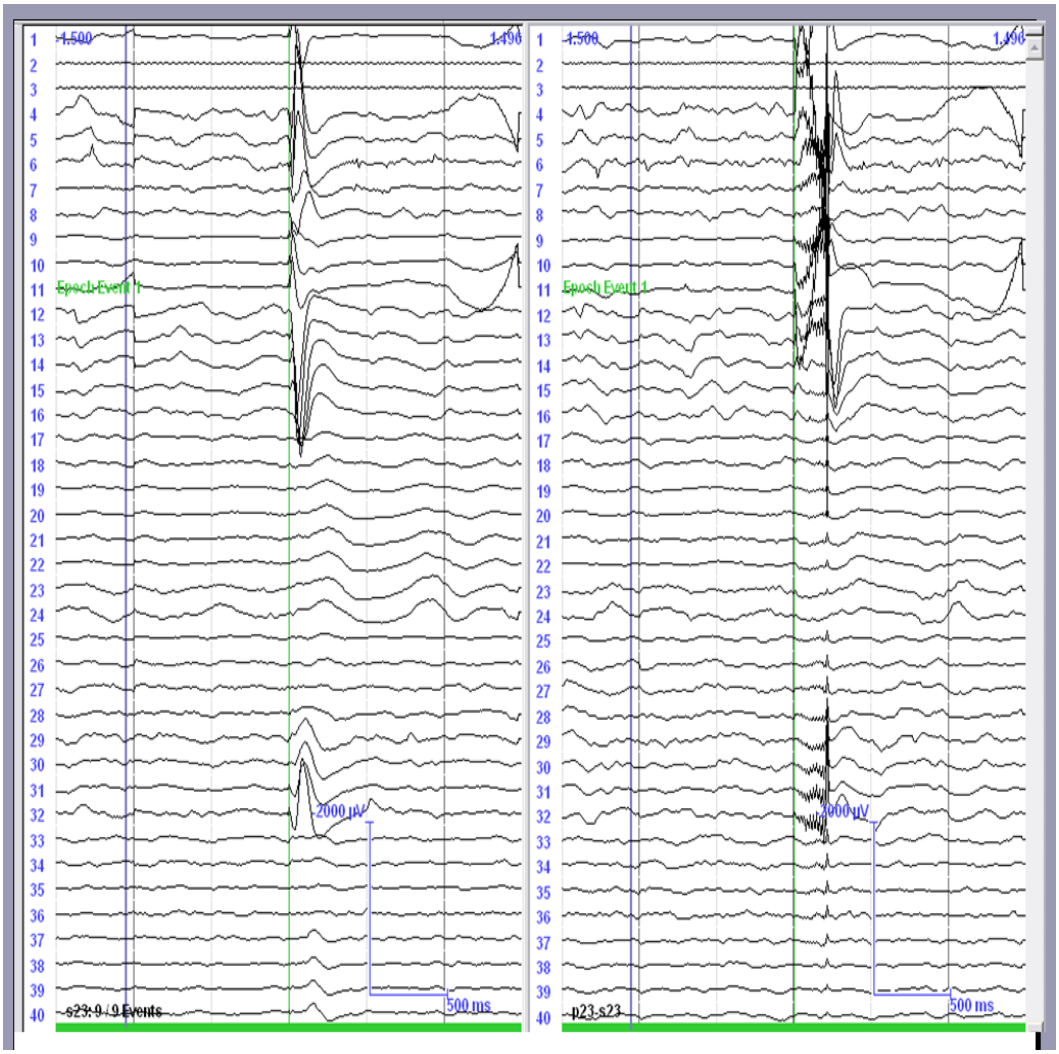

Figure 34. Patient 39. Suppression is seen in channels 8 and 37-40.

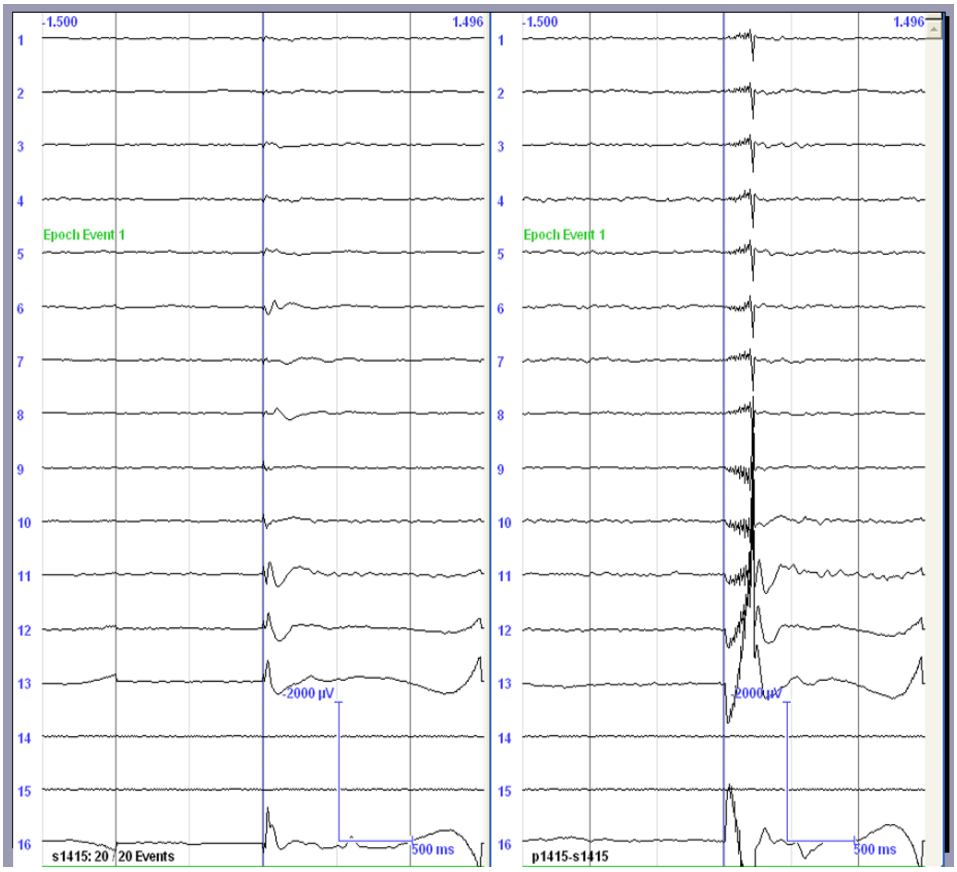

Figure 35. Patient 40. Suppression is seen in channels 6 and 8.

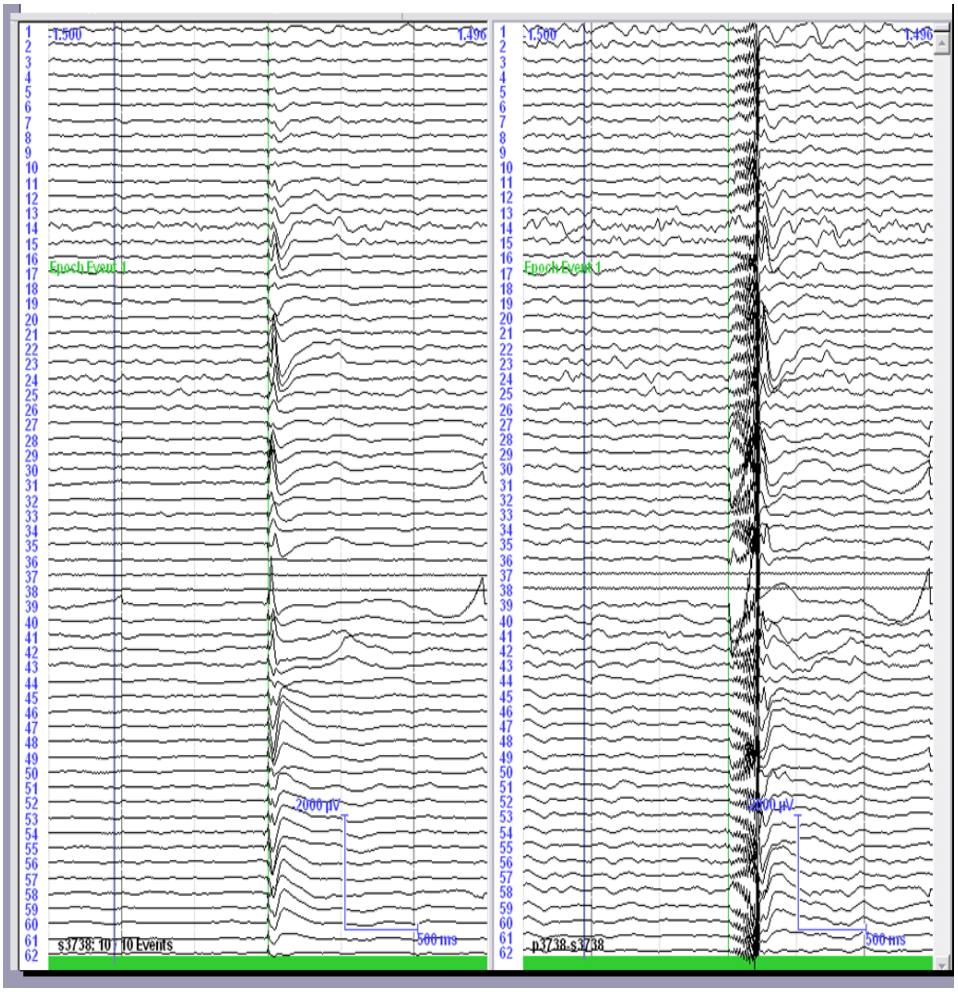

Figure 36. Patient 41. Suppression is seen in channel 34.

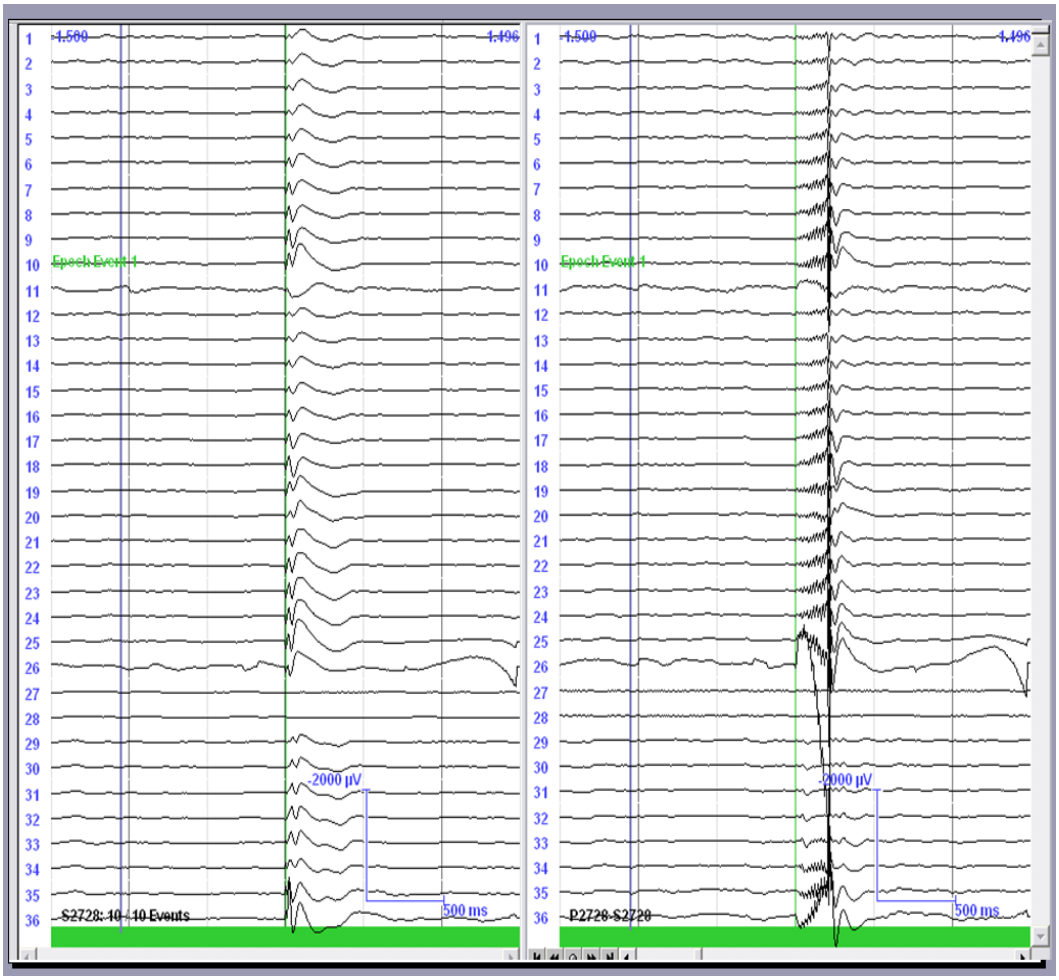

Figure 37. Patient 42. Suppression is seen in channels 29-32

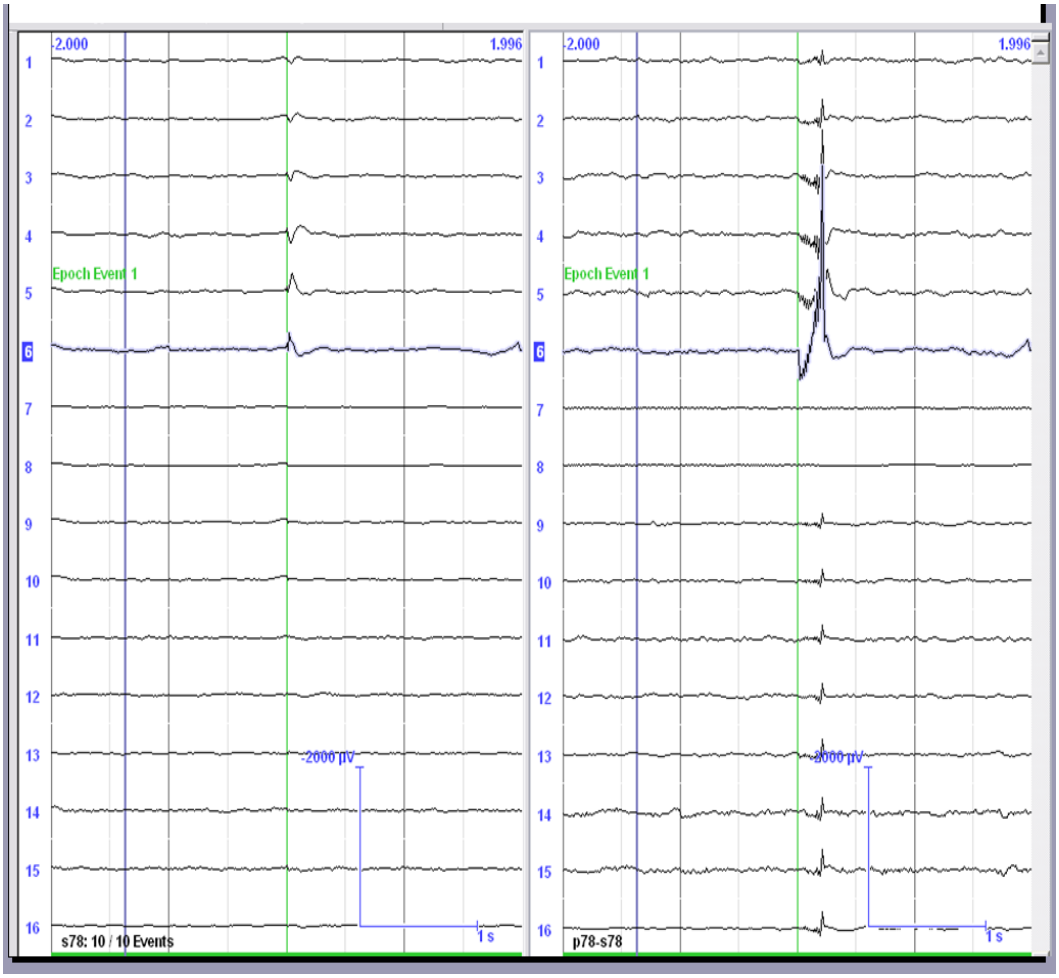

Figure 38. Patient 43. Suppression is seen in channels 1 and 2.

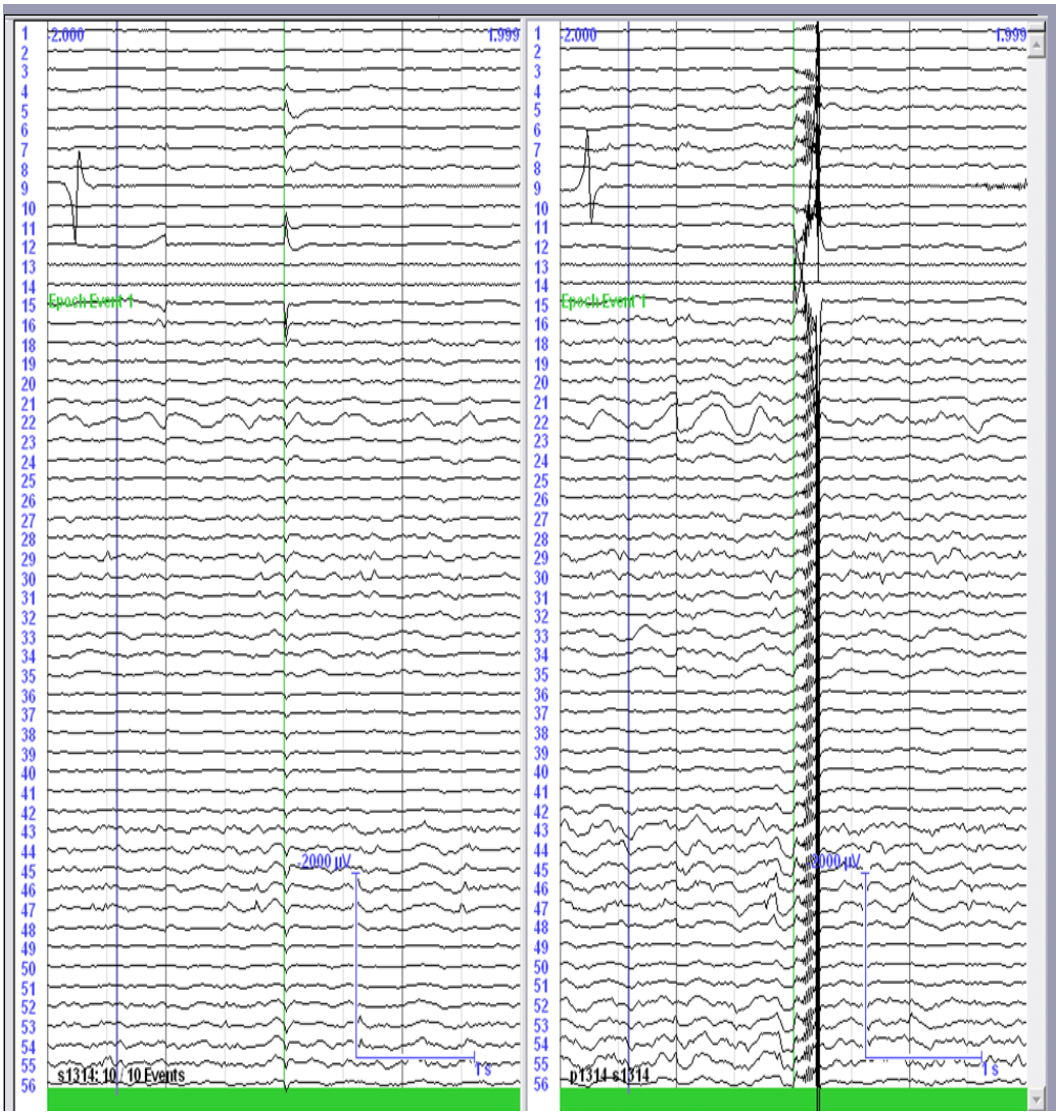

Figure 39. Patient 44. Suppression is seen in channels 31 and 32.

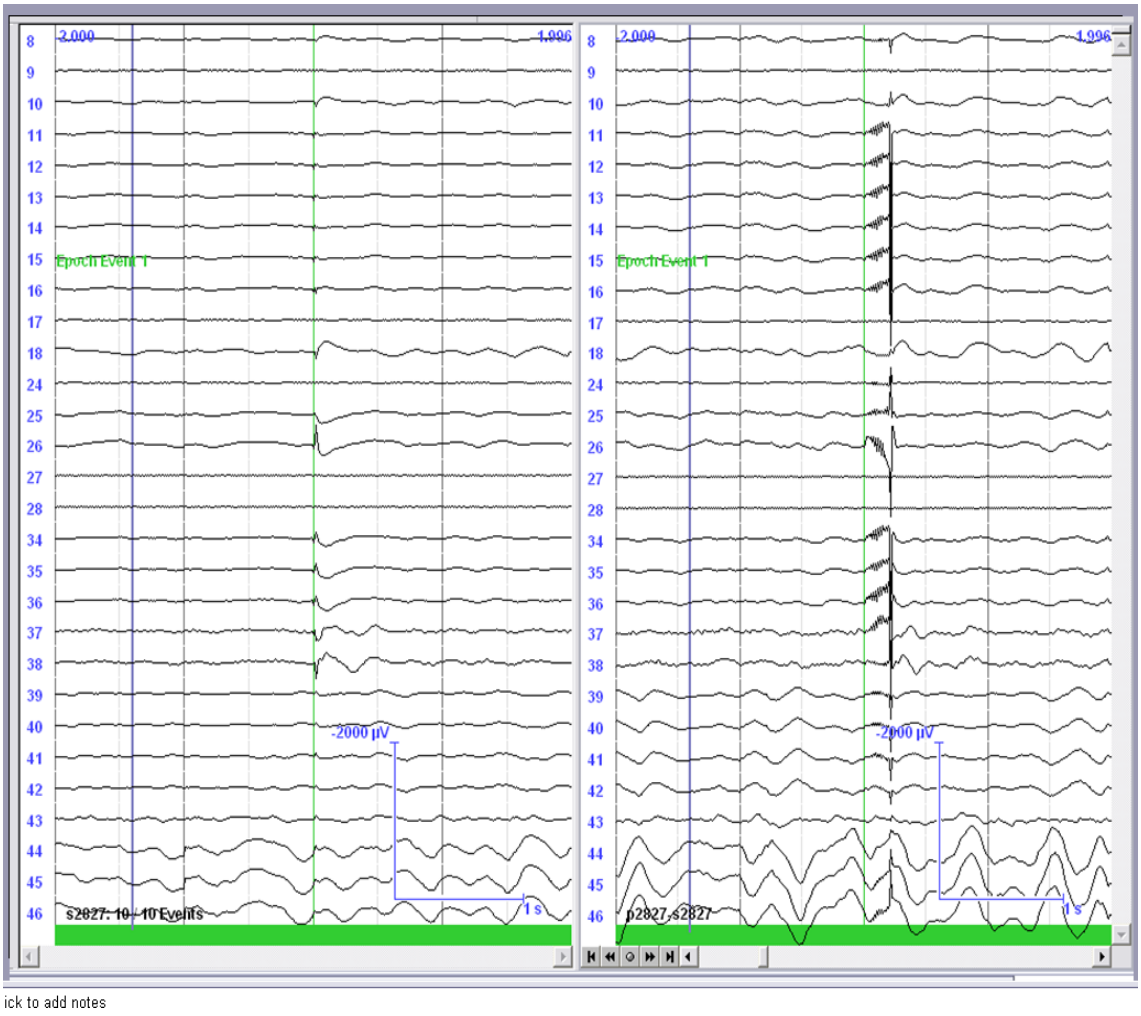

Figure 40. Patient 45. Suppression is seen in channel 26.

Figure 41. Patient 46. Suppression is seen in channels 15 and 16. Inconclusive SO patient.

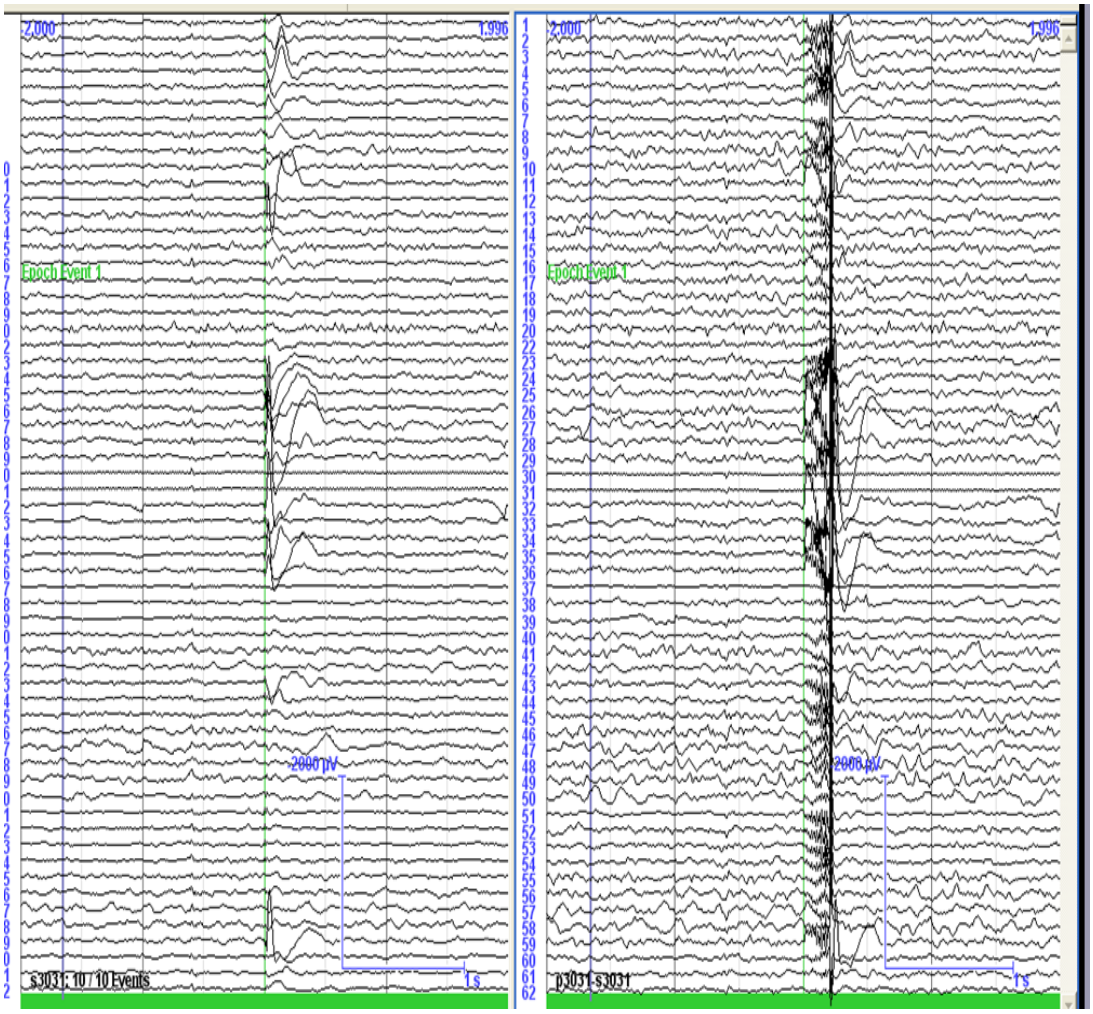

Figure 42. Patient 48. Suppression is seen in channels 23 and 24.

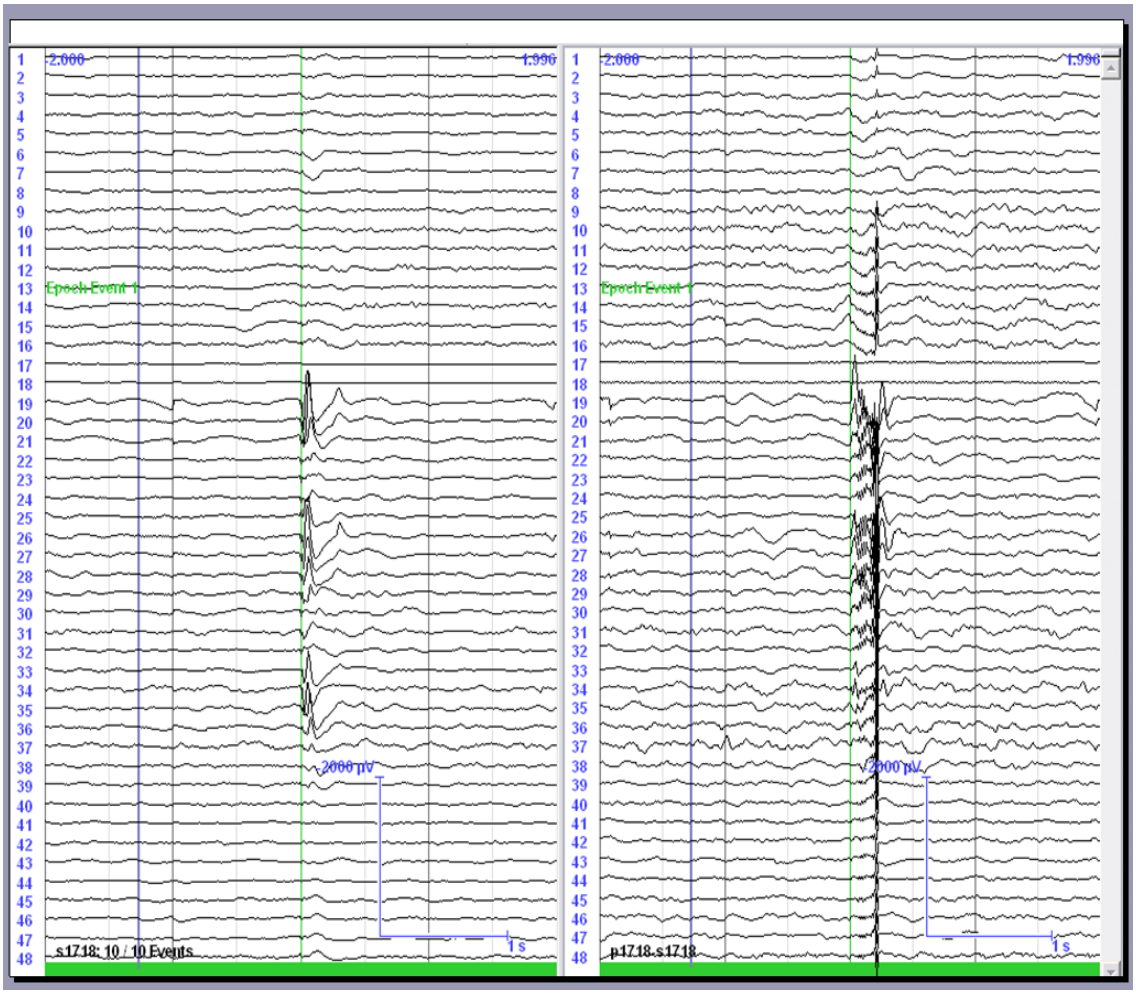

Figure 43. Patient 49. Suppression is seen in channels 28, 35, 36 and 45-48.

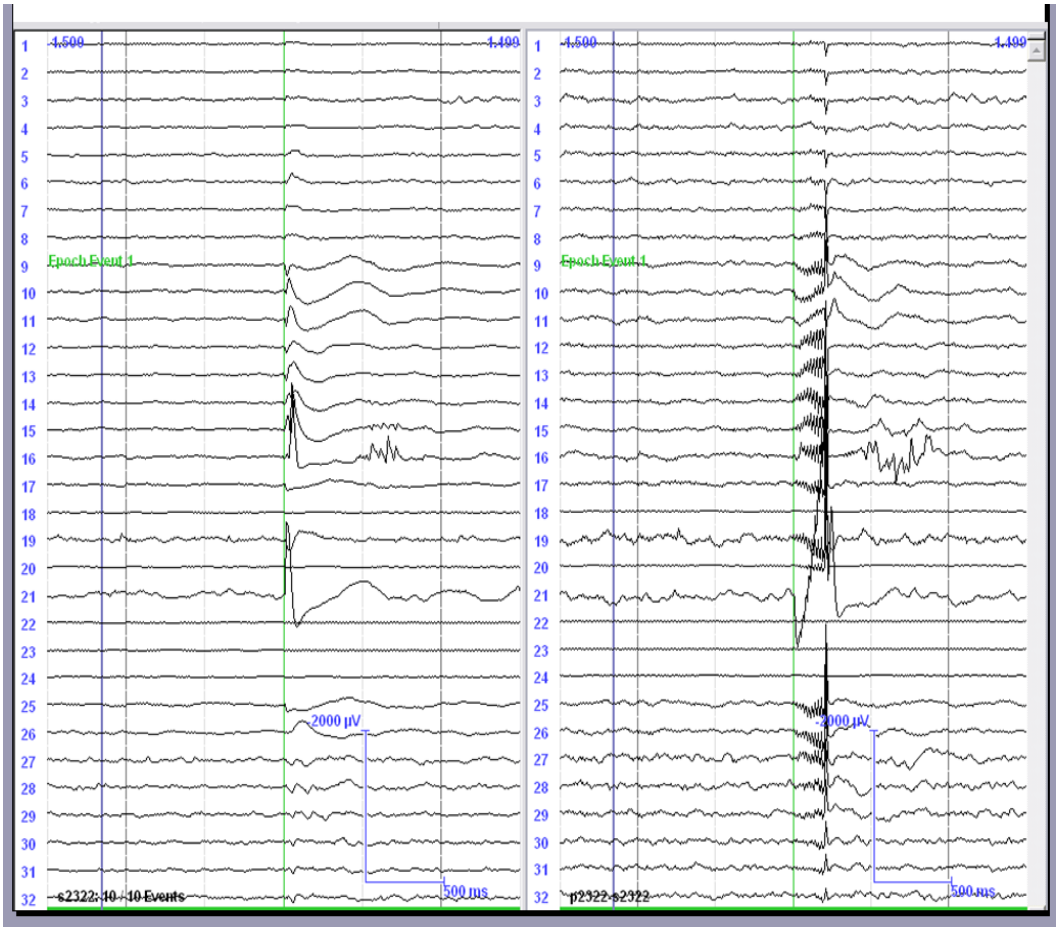

Figure 44. Patient 51. Suppression is seen in channels 12-15.

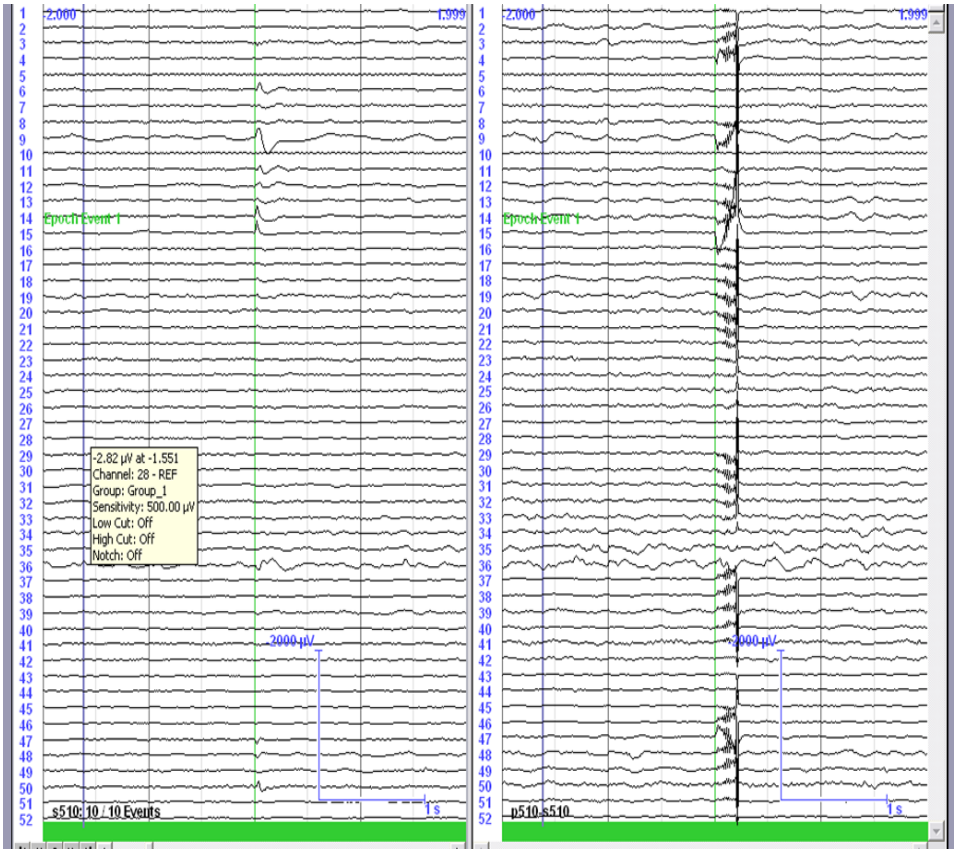

Figure 45. Patient 52. Suppression is seen in channels 6 and 50.

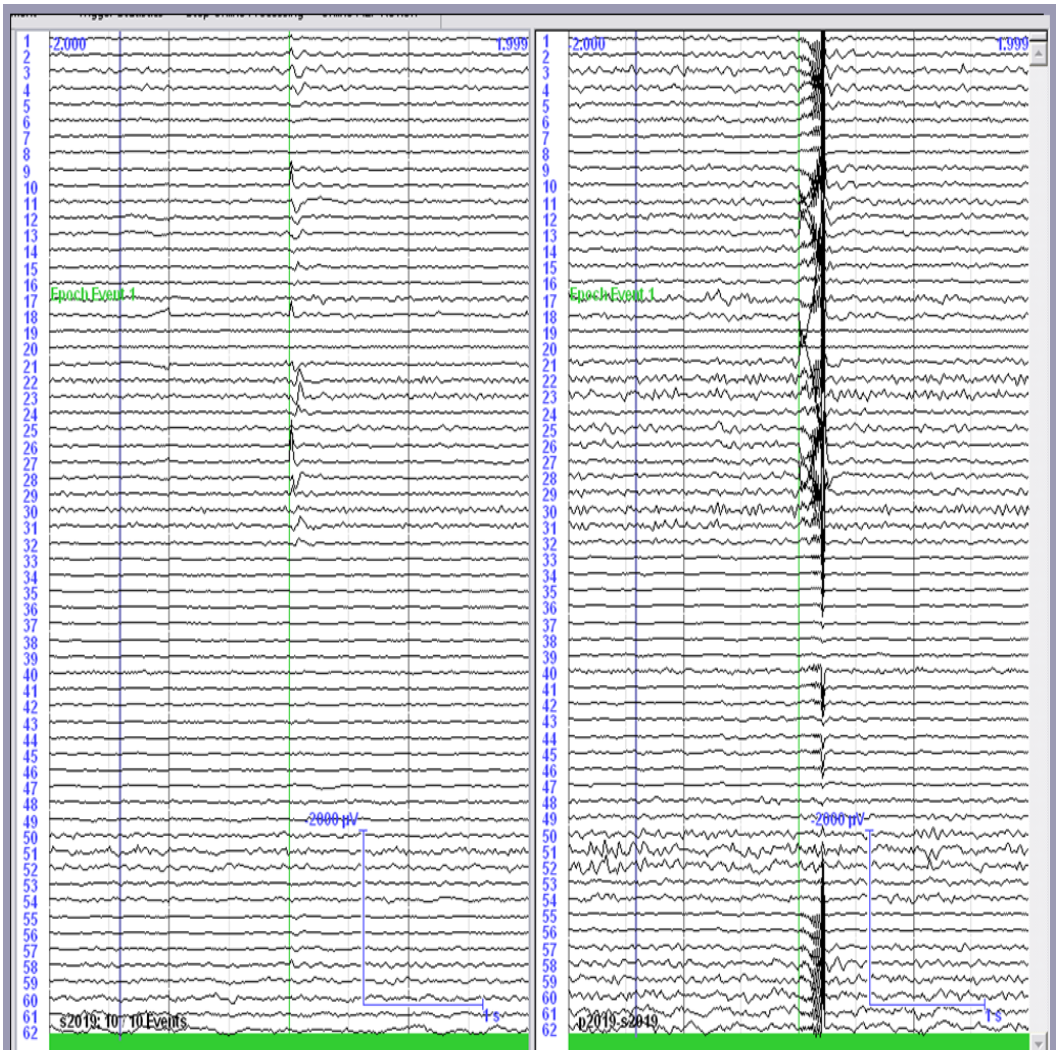

Figure 46. Patient 53. Suppression is seen in channel 31.

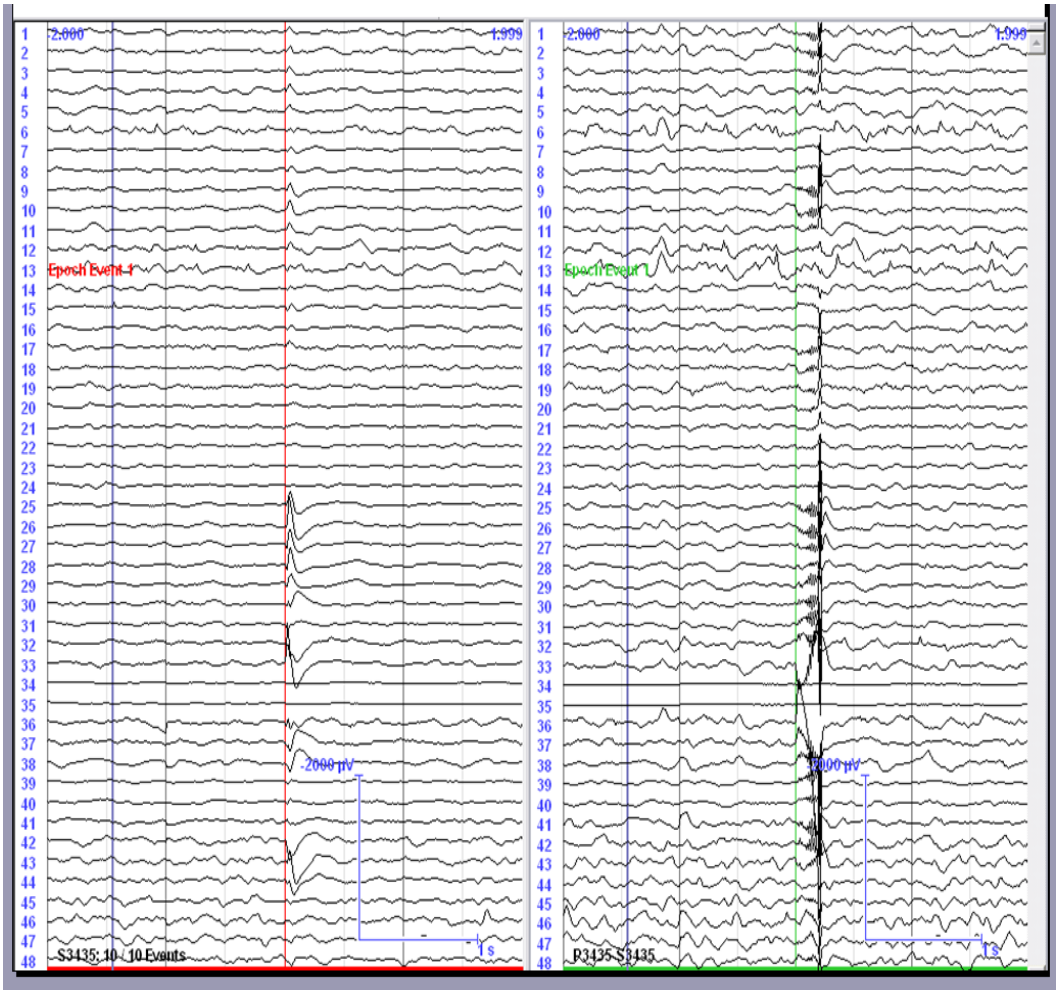

Figure 47. Patient 54. Suppression is seen in channels 29, 37 and 38.

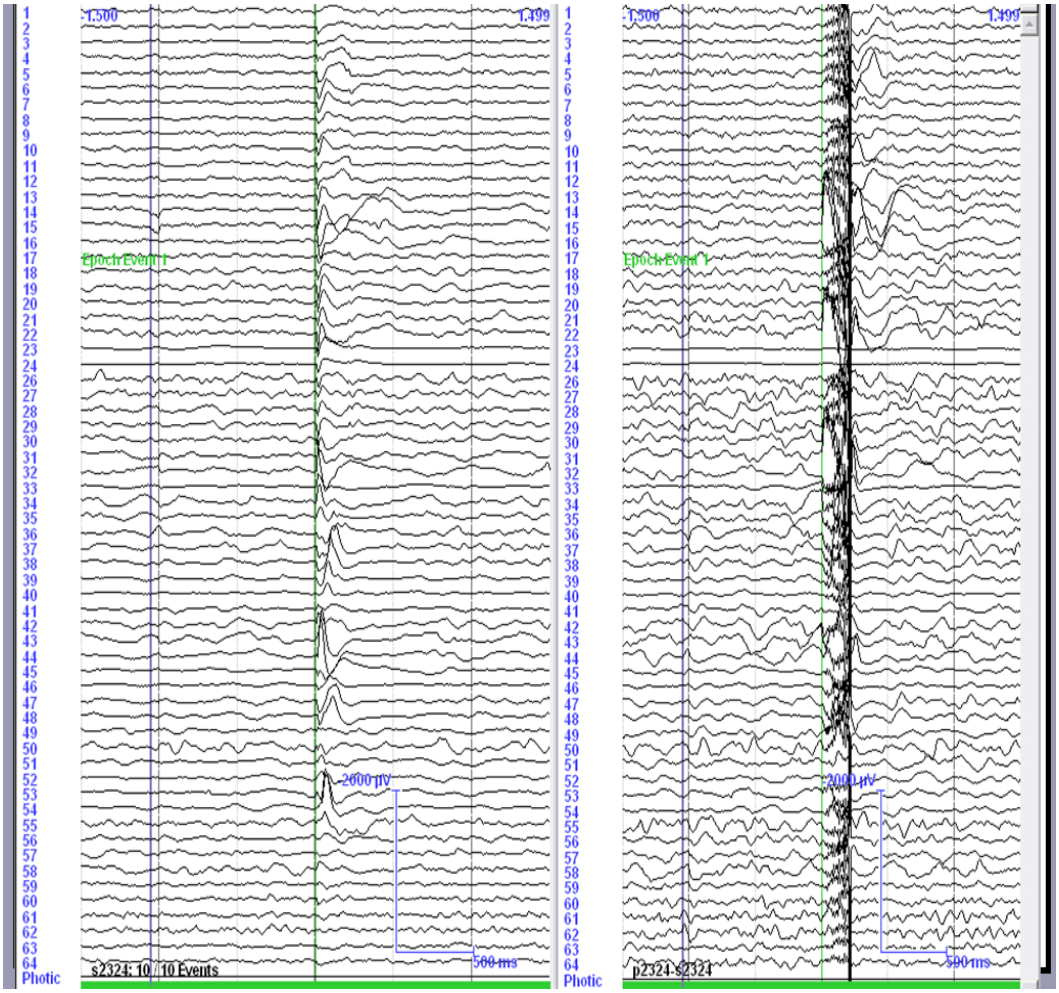

Figure 48. Patient 27. Suppression is seen in channels 8, 9, 11, 36-40, 46, 47, 53 and 54.

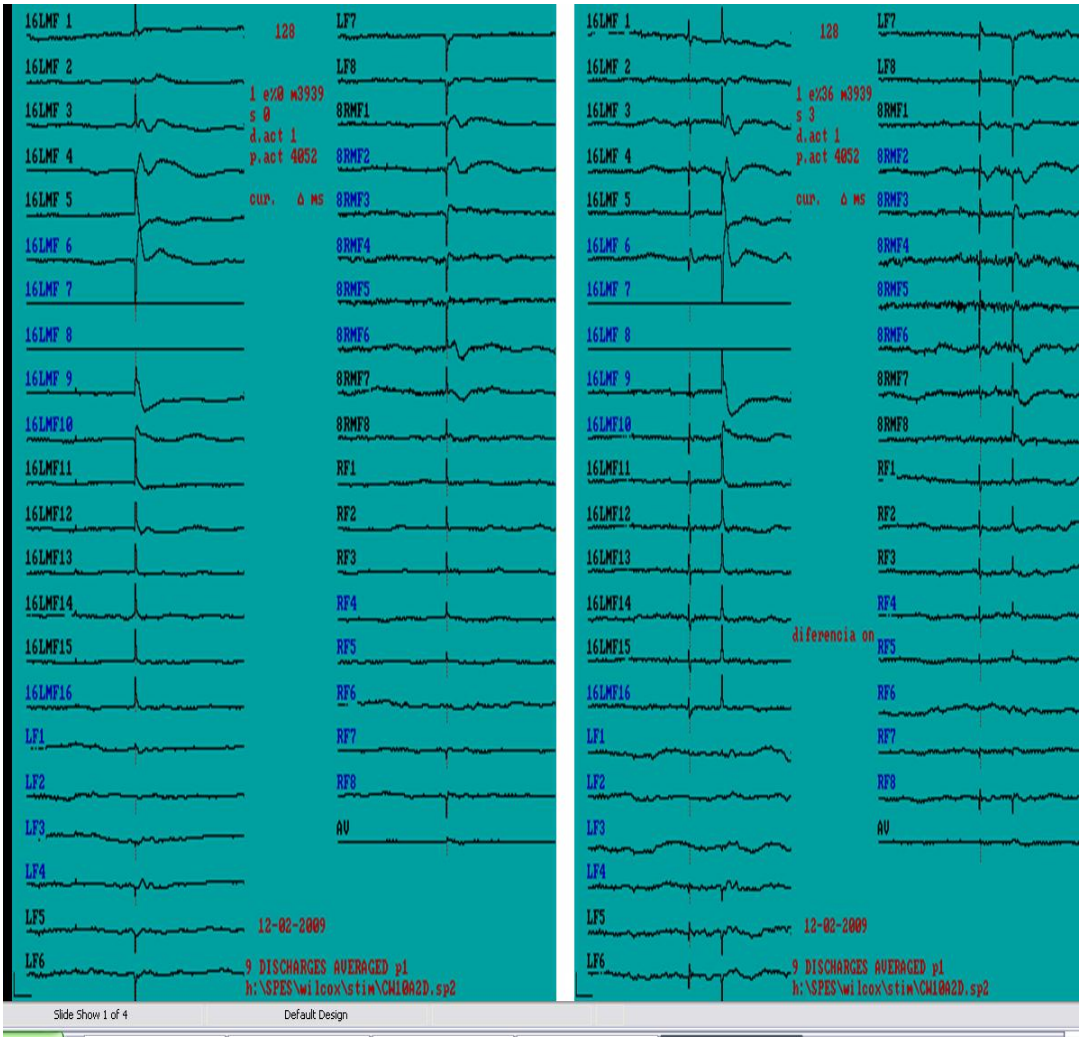

Figure 49. Patient 58. Suppression is seen in channel RMF1

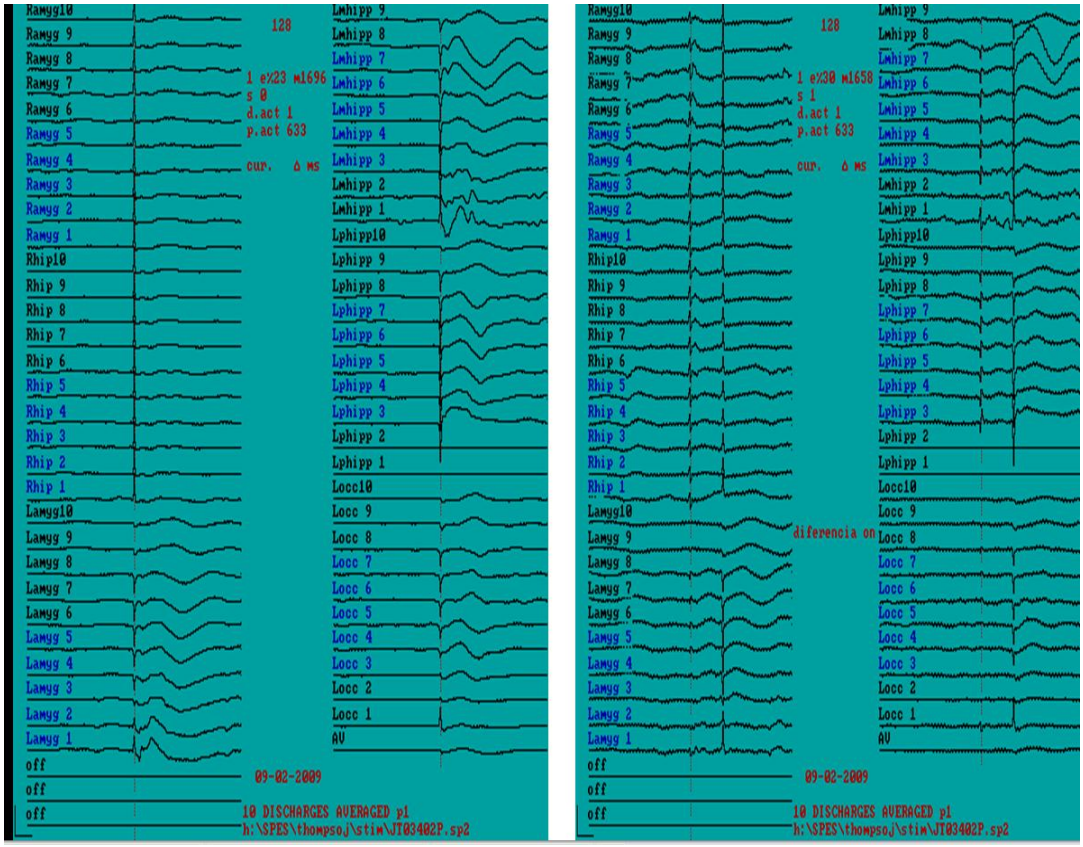

Figure 50. Patient 59. Suppression is seen in channel Lamg 2 -1 (left amygdala).

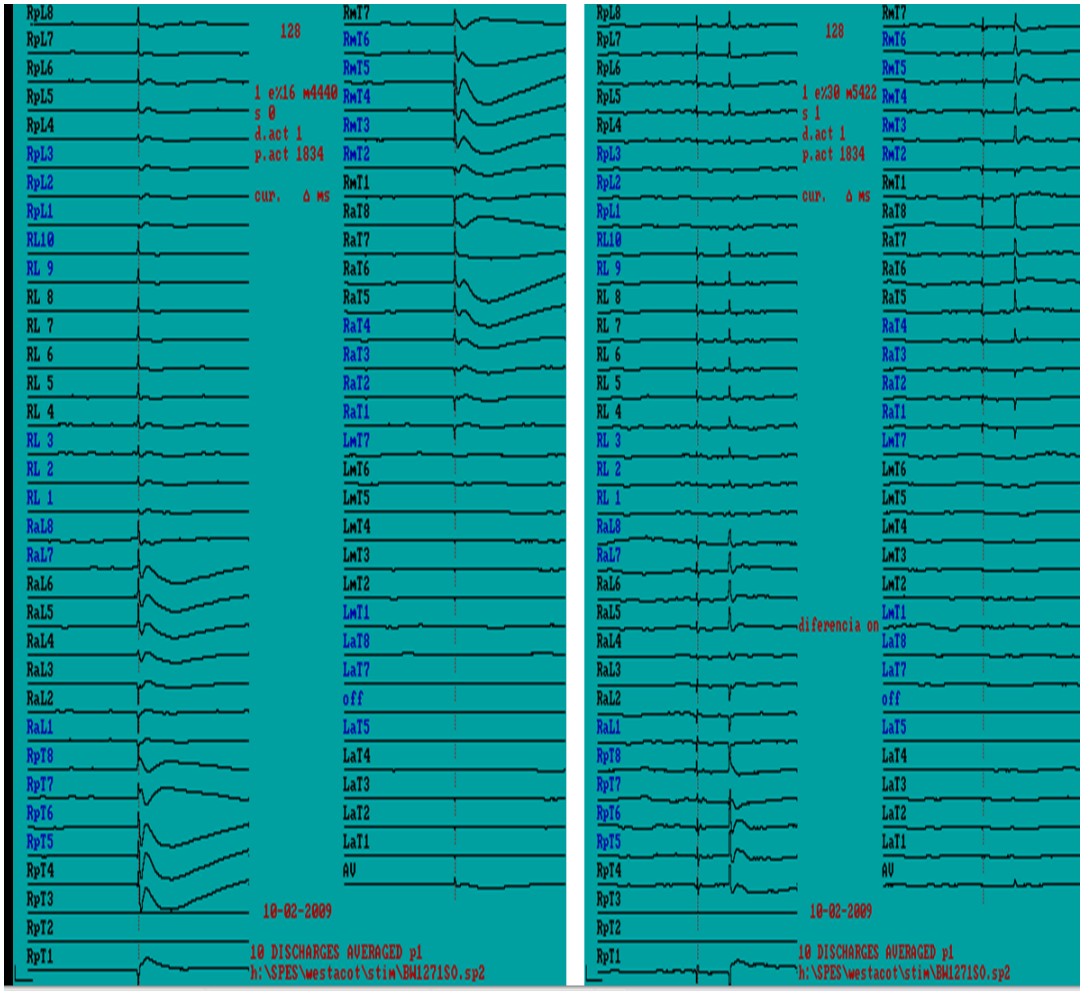

Figure 51. Patient 61. Suppression is seen in channels RaL7-4 (right anterior lesional), RmT 7-2 (right mid-temporal) and RaT 6-2 (right anterior temporal).

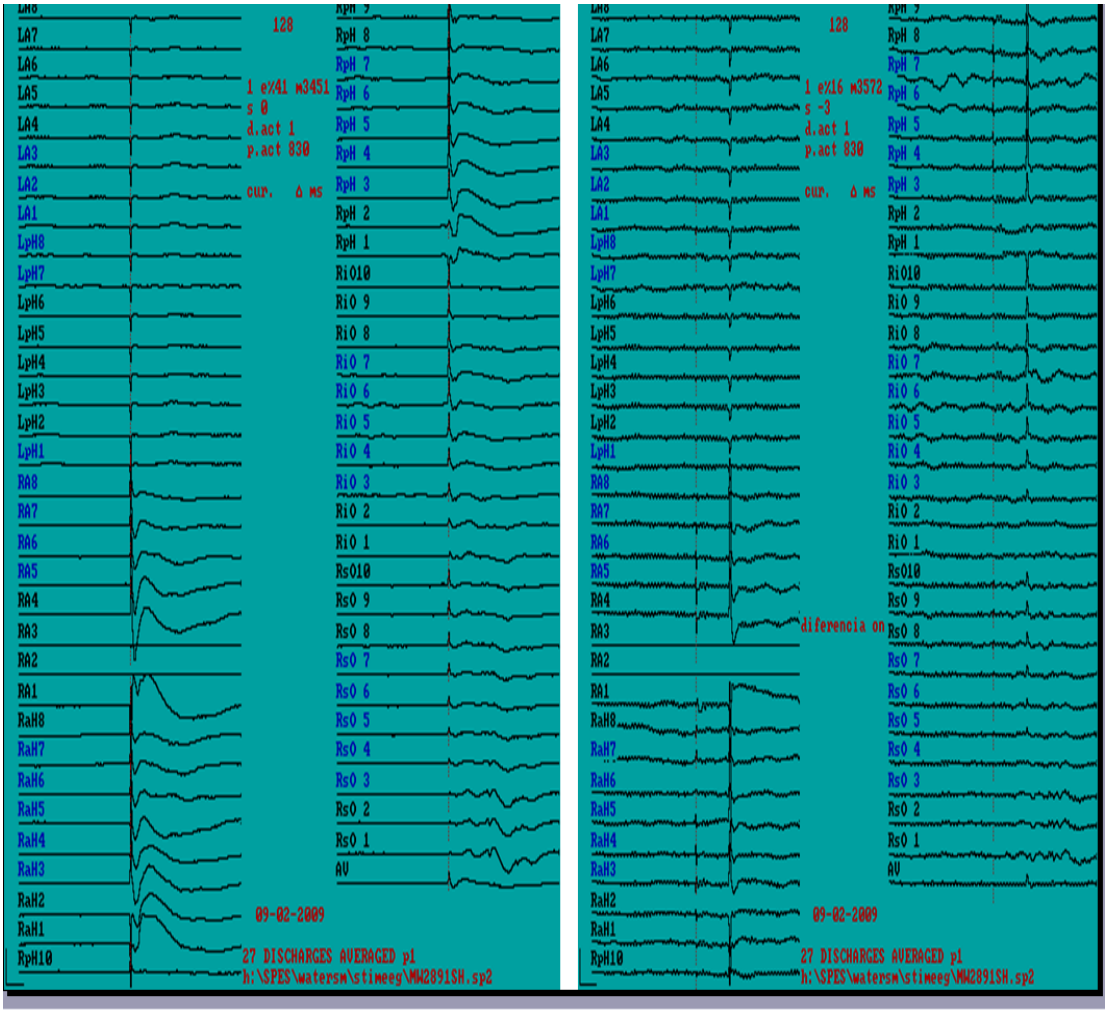

Figure 52. Patient 60. Suppression is seen in the entire RaH (right anterior hippocampus) and RpH 7-2 (right posterior hippoccampus) as well in in RiO 3-1 (right inferior occipital) and RsO 4-1 (right superior occipital).

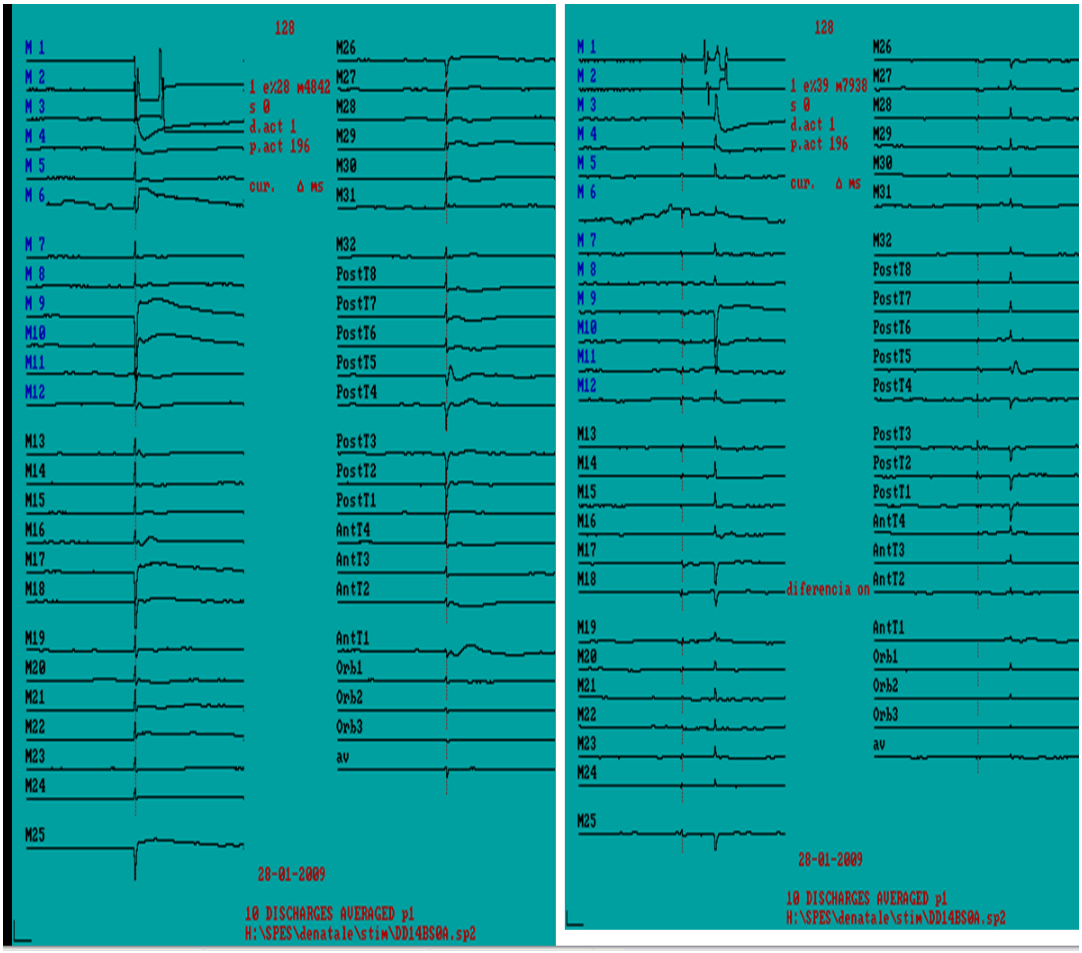

Figure 53. Patient 62. Suppression is seen in AntT 1 (anterior temporal). Inconclusive SO patient.

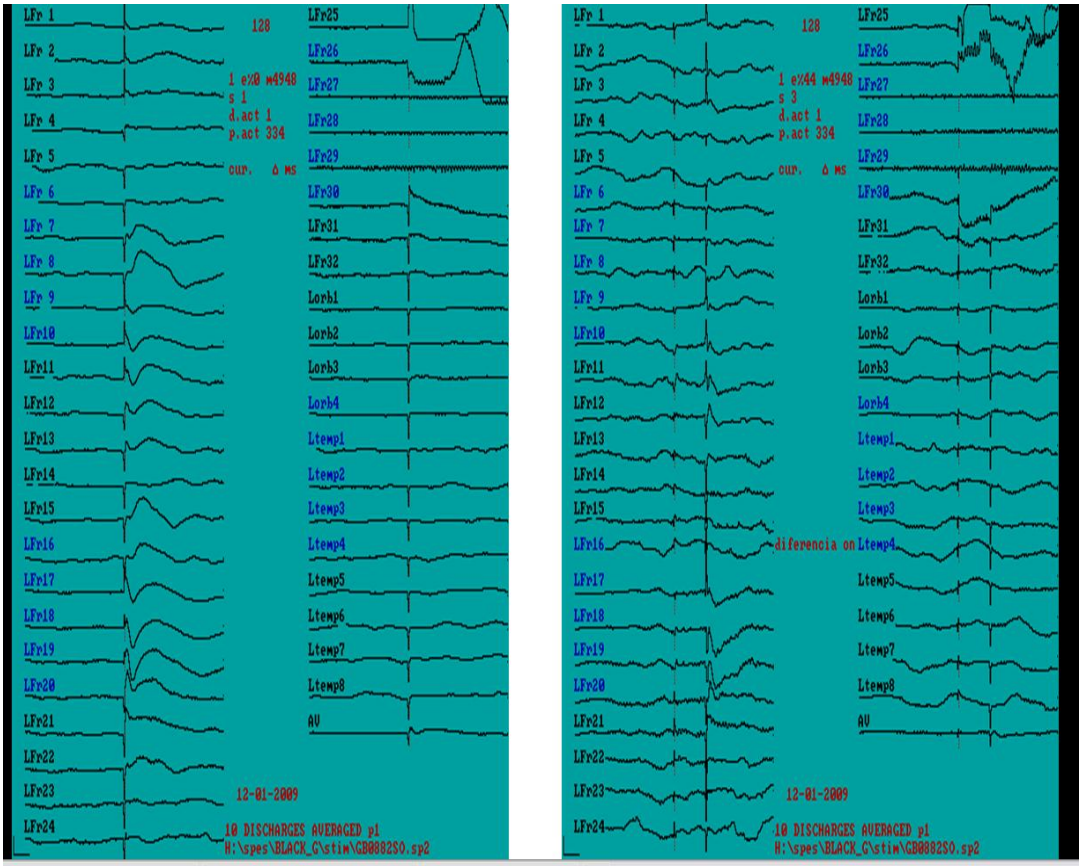

Figure 54. Patient 63. Suppression is seen in LF 7-9 (left frontal).

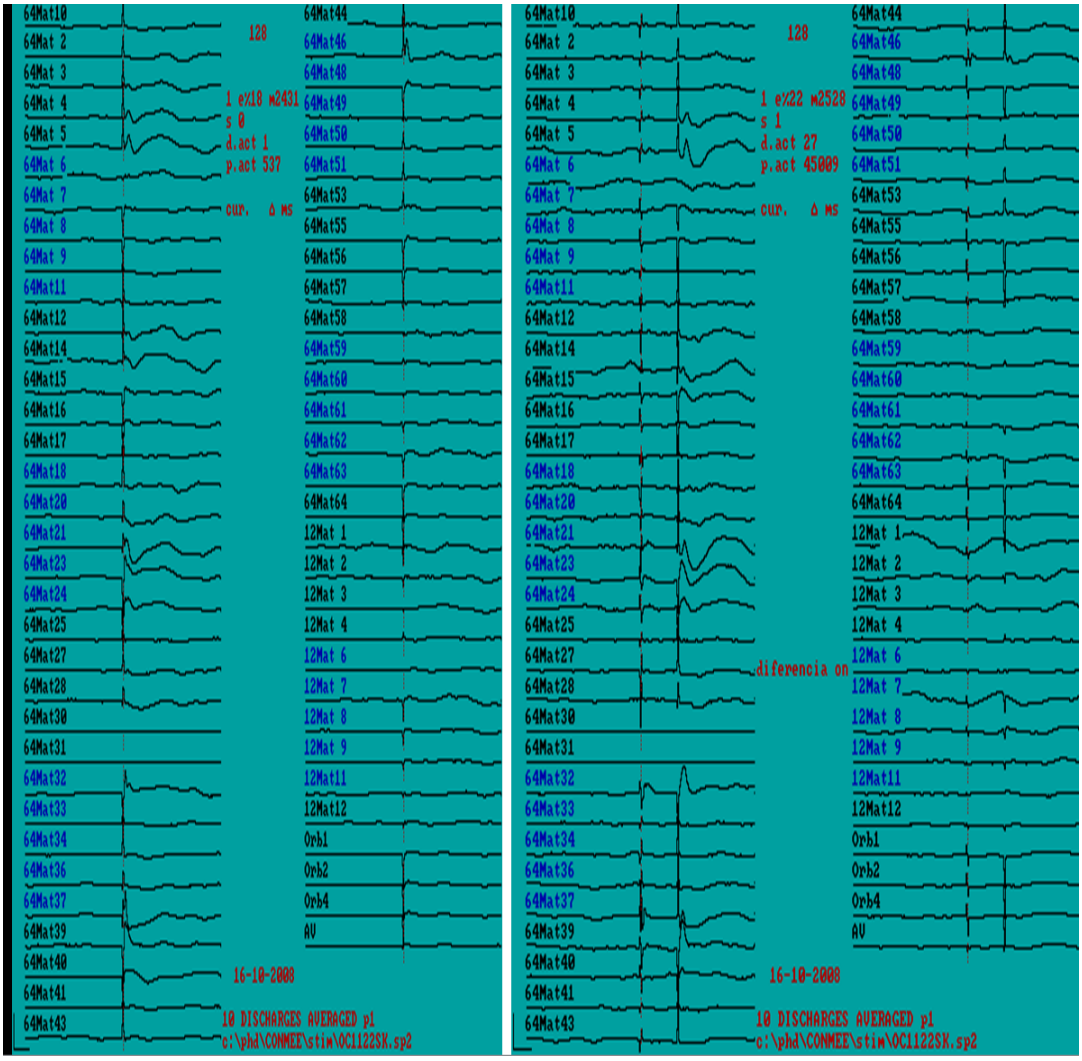

Figure 55. Patient 64. Suppression is seen in the 64Mat40.

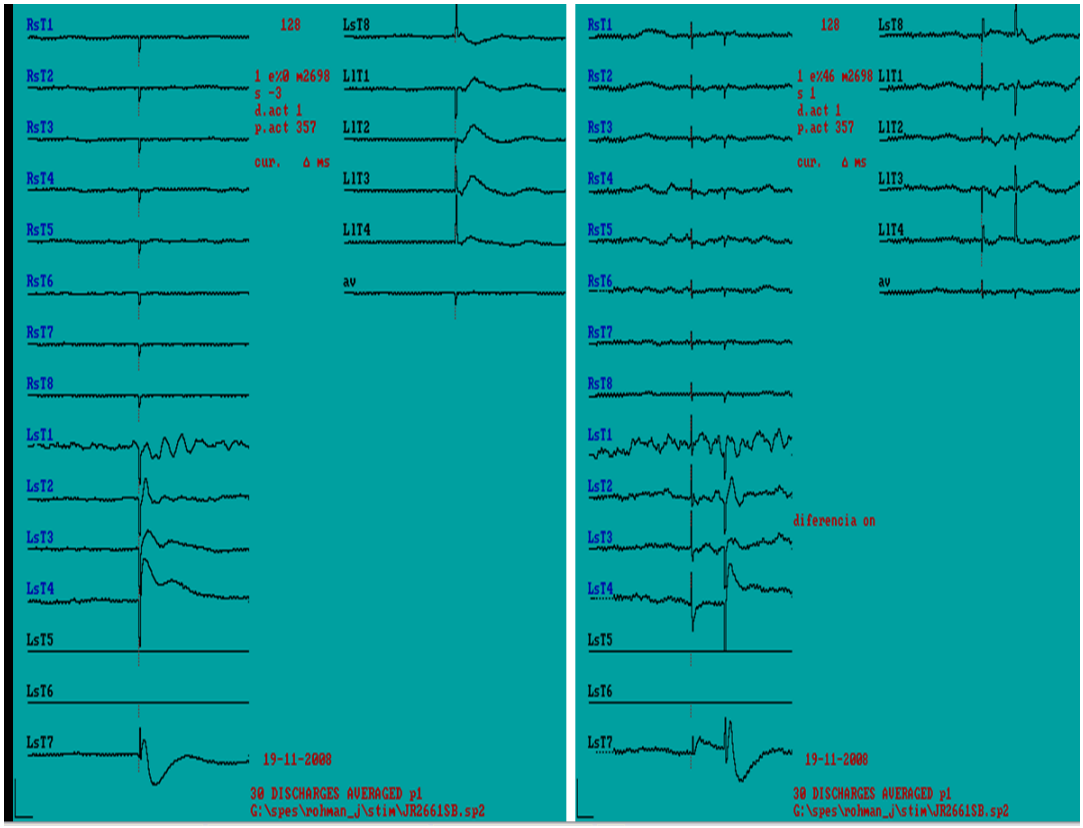

Figure 56. Patient 71. Suppression is seen in the LIT2-4 (left inferior temporal).

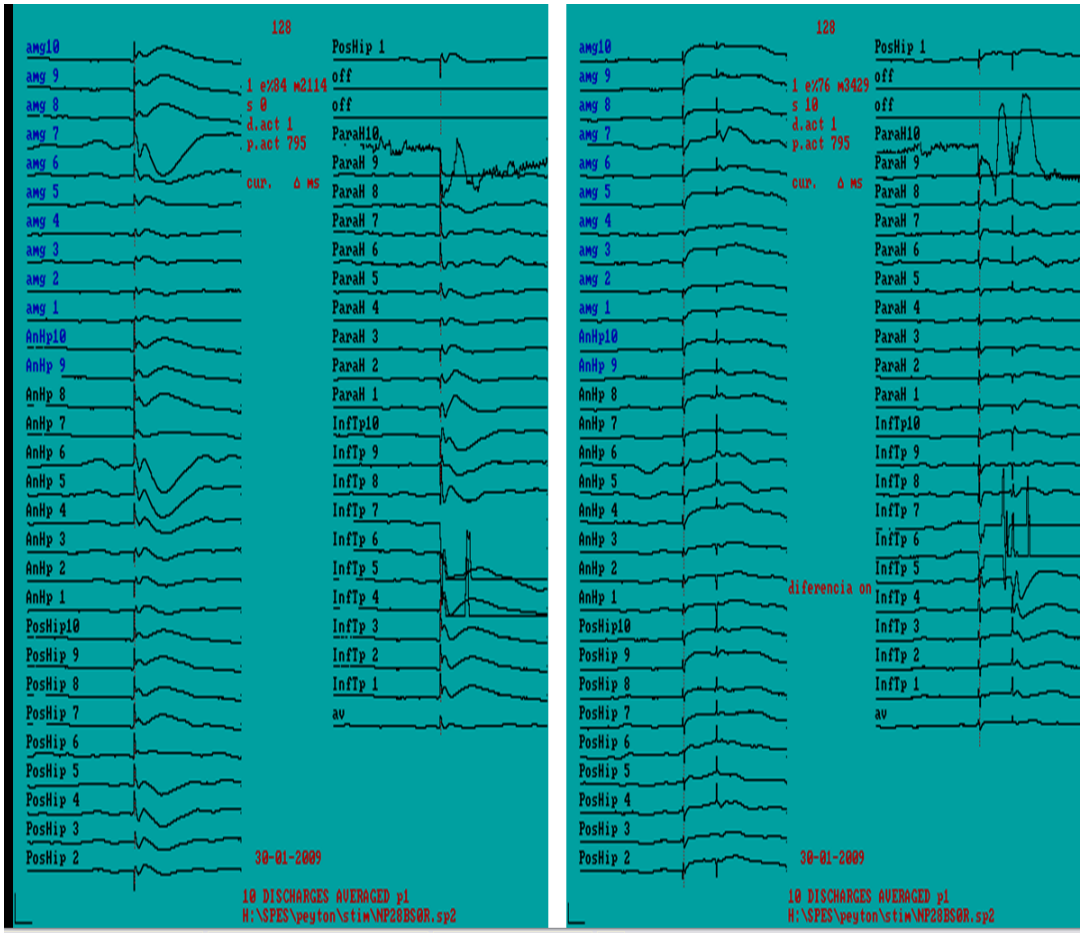

Figure 57. Patient 69. Suppression is seen in the Amg 4-1, AntHip 6-1 (anterior hippocampus), PostHip 4-1 (posterior hippocampus) and ParaHip 4 and 3 (Parahippocampus).

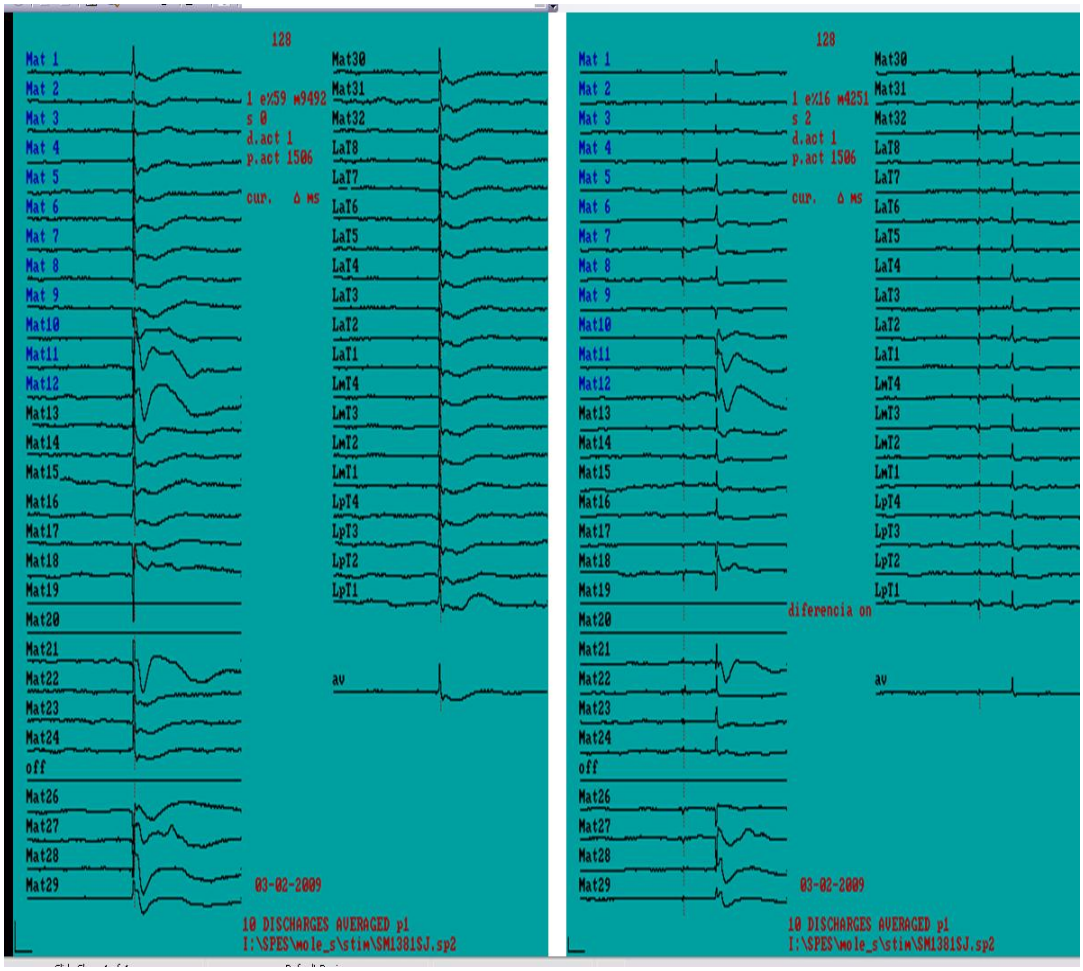

Figure 58. Patient 66. Suppression is seen in LpT 1 (left posterior temporal).

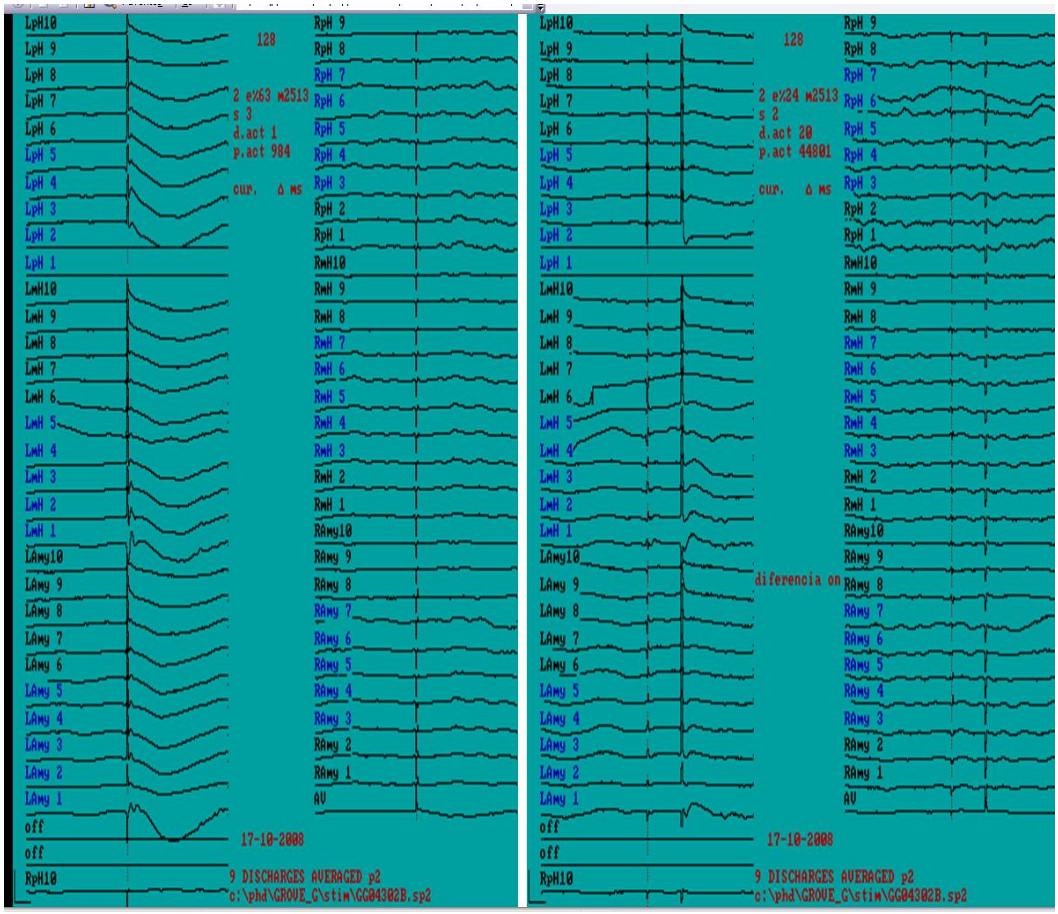

Figure 59. Patient 72. Suppression is seen in LAmg 1 (left amygdala).

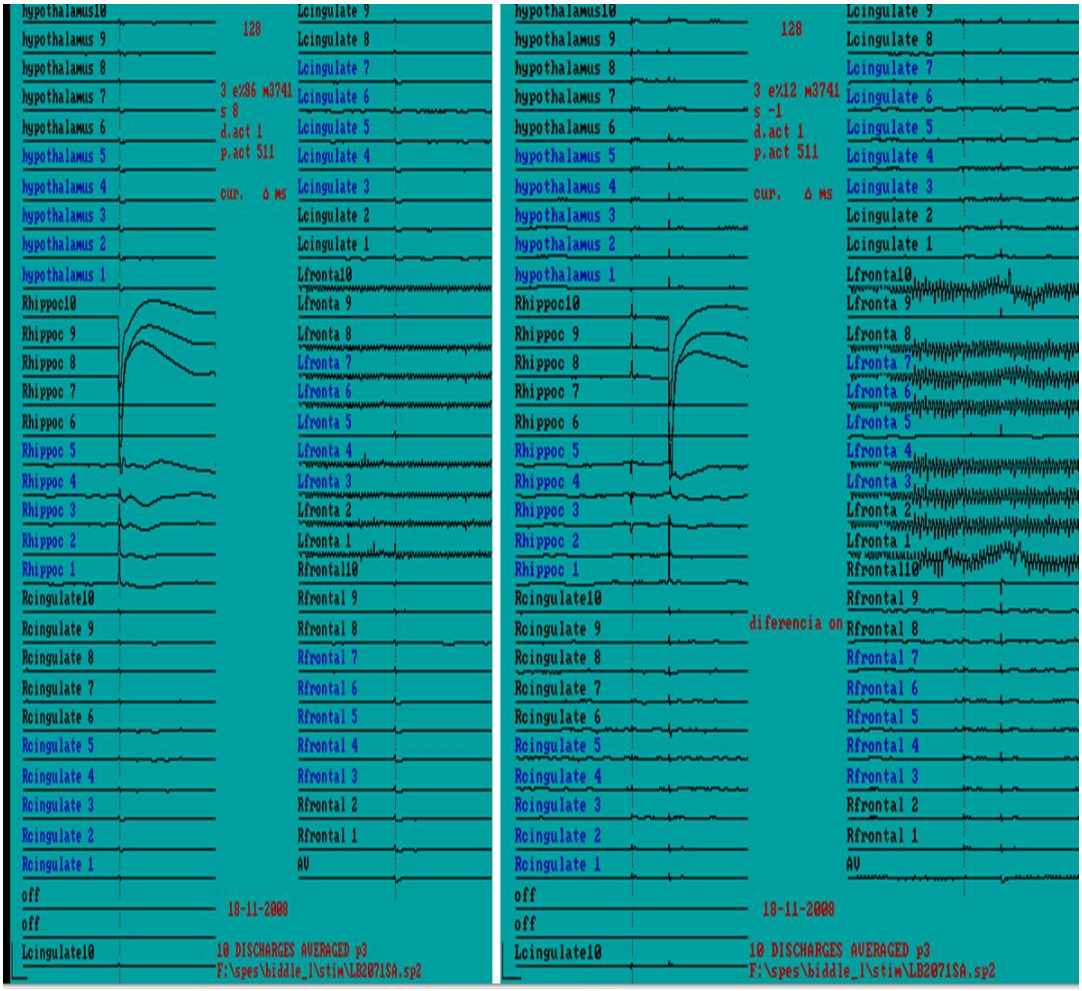

Figure 60. Patient 74. Suppression is seen in RH 3-1 (right hippocampus).

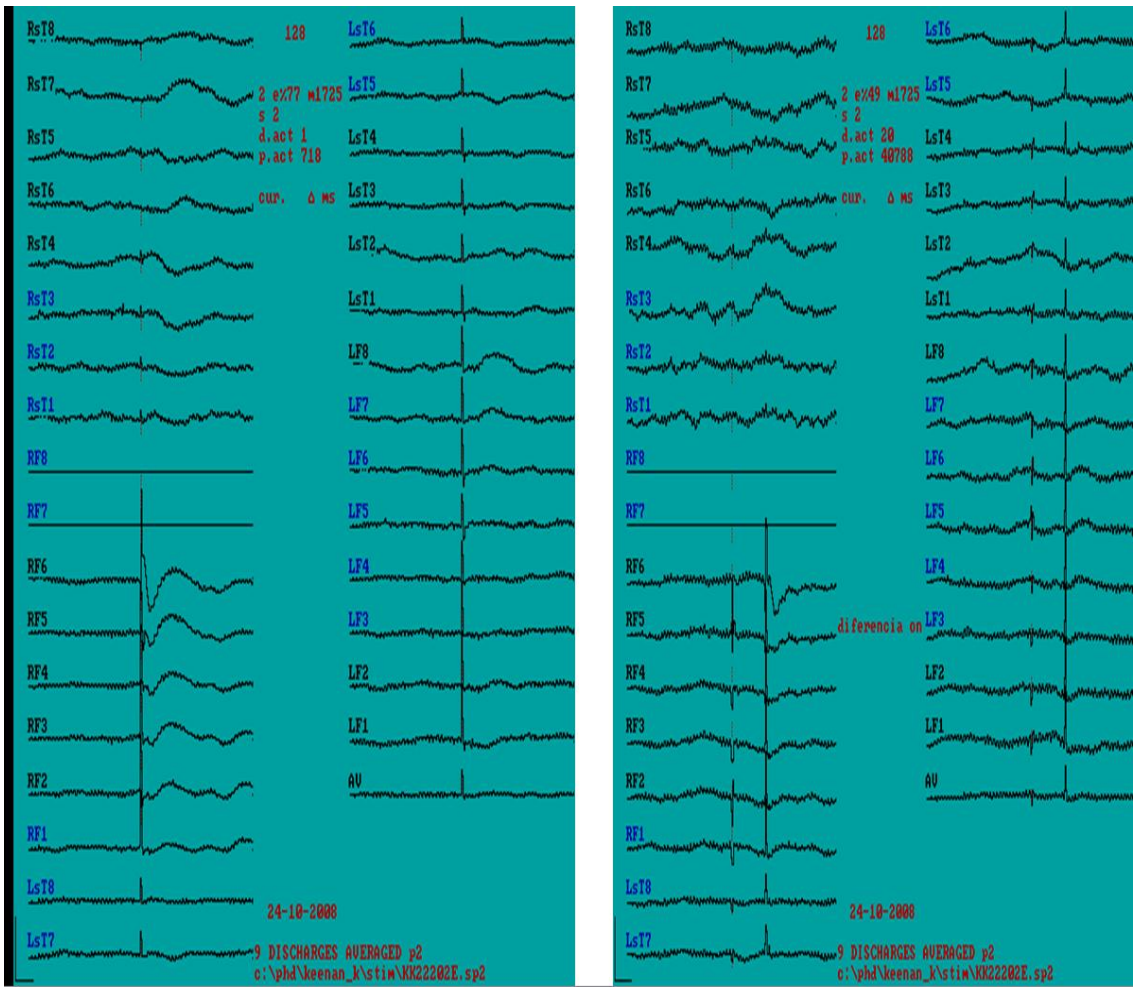

Figure 61. Patient 75. Suppression is seen in RF 5-2 (right frontal).

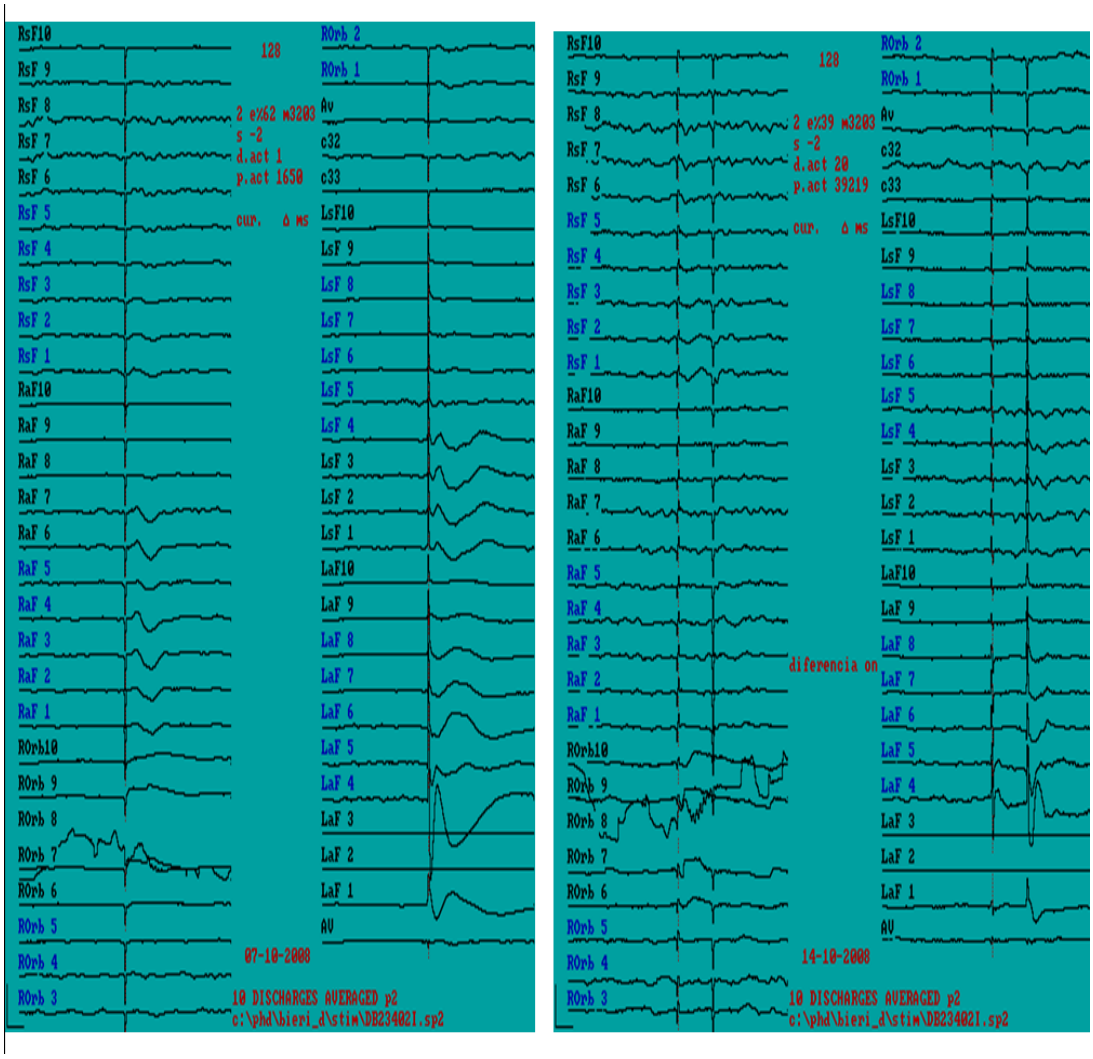

Figure 62. Patient 76. Suppression is seen in RaF 7-1 (right anterior frontal) and LsF 4-1 (left superior frontal).

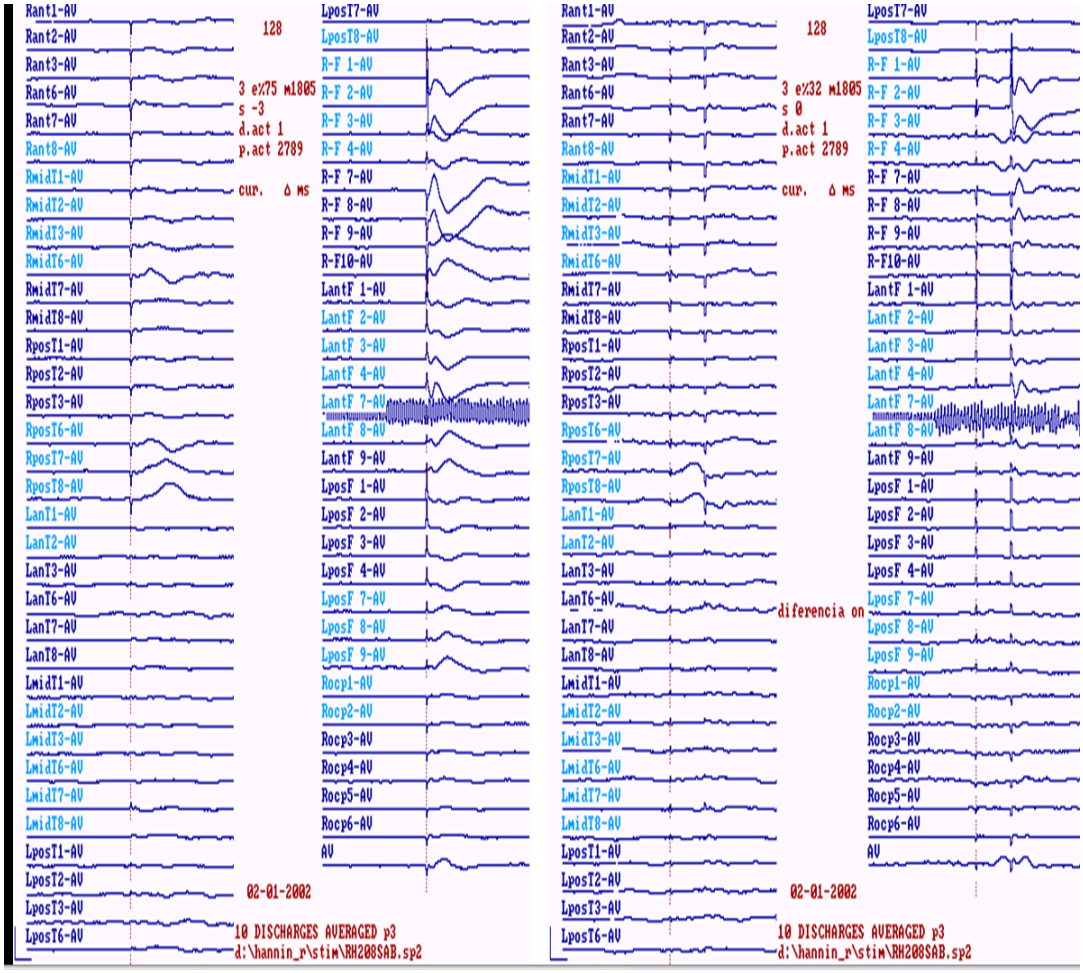

Figure 63. Patient 78. Suppression is seen in Rpt 6-8 (right posterior temporal).

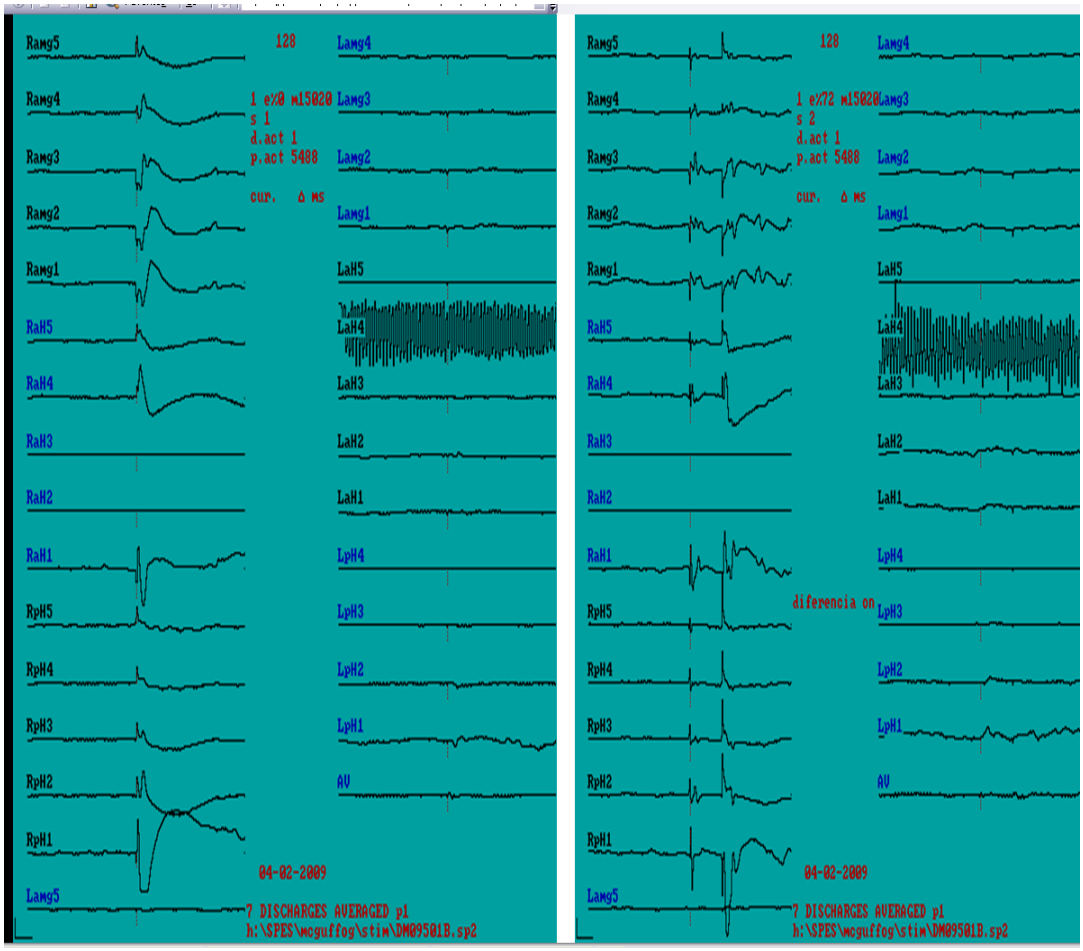

Figure 64. Patient 80. Suppression is seen in Ramg 5, 4 (right amygdala).

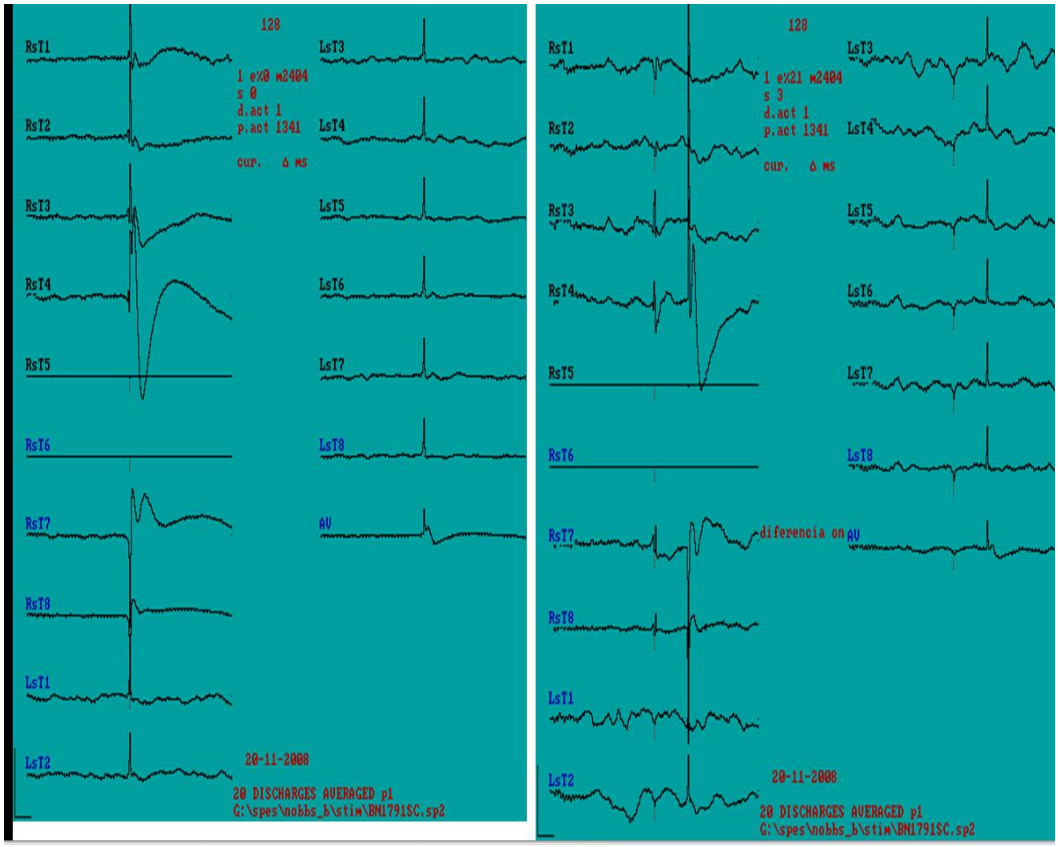

Figure 65. Patient 81. Suppression is seen in RsT 3 (right sub-temporal).

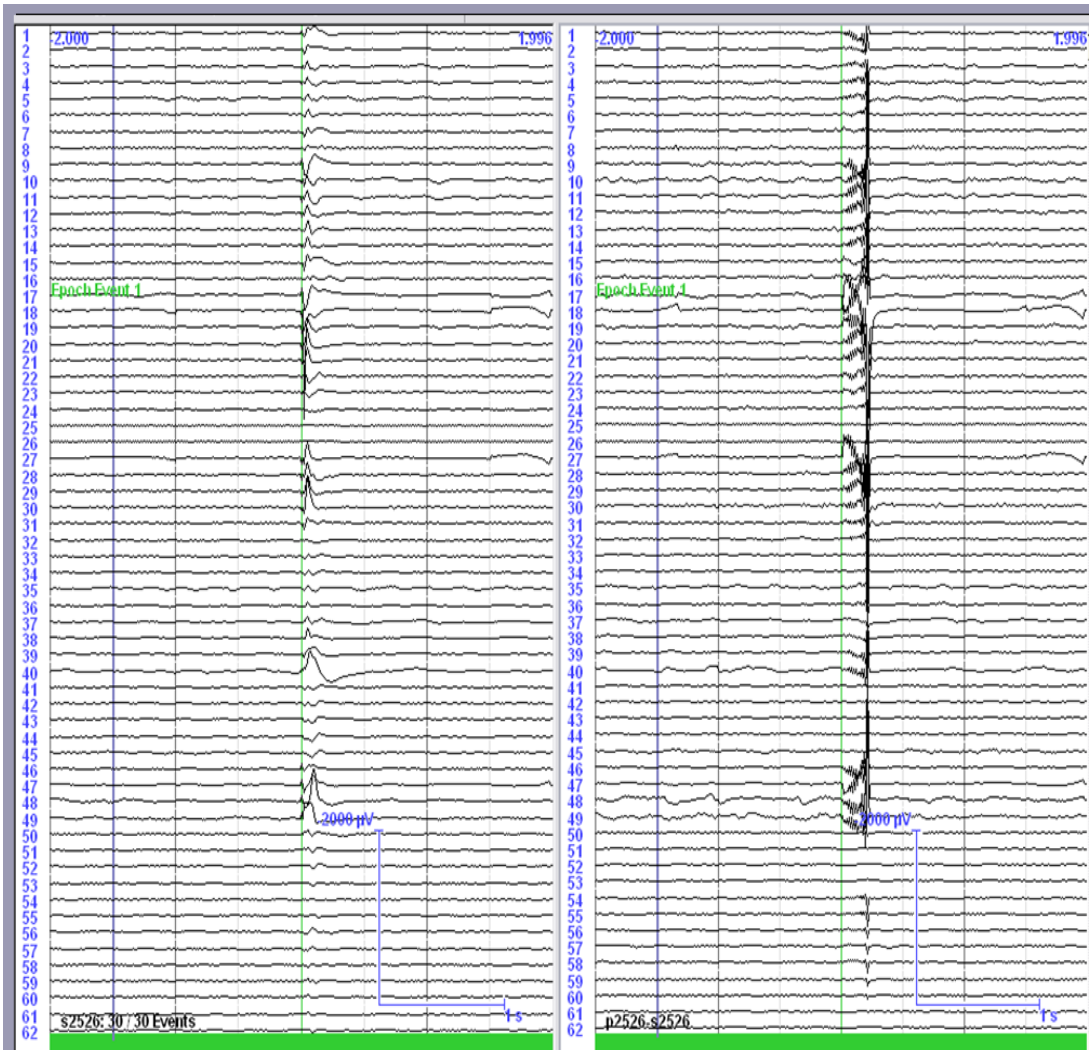

Figure 66. Patient 55. Suppression is seen in channels 1-7, 9-15, 19-22, 28-31 38-40 and 47-49.

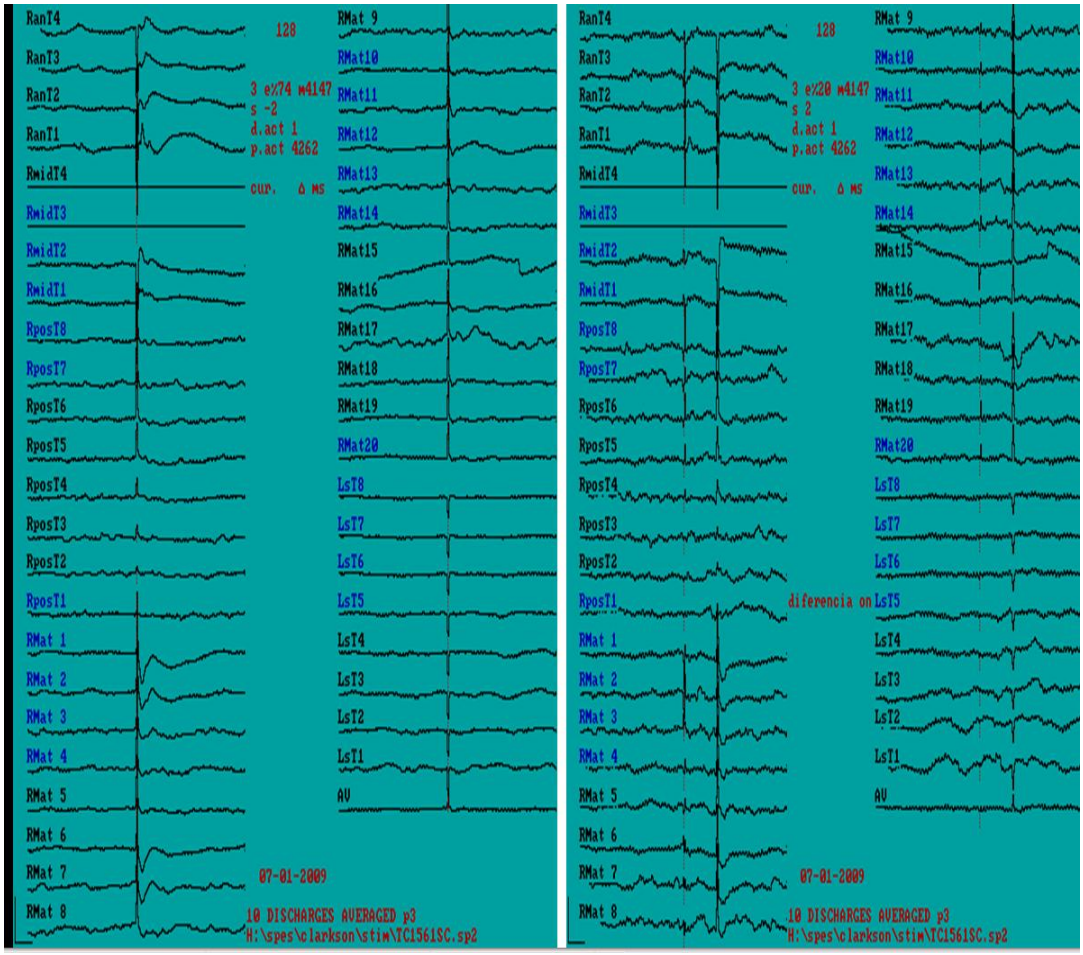

Figure 67. Patient 77. Suppression is seen in RaT 4-1 (right anterior temporal).

APPENDIX II

APPENDIX 2-THE ALGORITHM

```
function [] = Master_v10()
% A. DATA LOADING

channel_list = [];
cd('/Users/ae301/Desktop/Projects 2011/EEG Data - Kings/ASCII/RESULTS/ASCII
FILES');
load('settings.mat');


for k =2%1:size(folder_list,1) %GO INTO EACH FOLDER

 cd(['ASCII ' char(folder_list{k,1})]); %GOES INTO THE FOLDER
 %if exist('Figure1_spes_ppes','dir') == 0 %CREATE FIGURES DIRECTORY
 mkdir Figure1_spes_ppes
 mkdir Figure2_histogram_old
 mkdir Figure2_histogram_new


 mkdir Figure3_final_plot_new
 mkdir Figure3_final_plot_old
 %end

 d1 =dir('*P*-S*_*.asc'); %SEARCHES FOR PPES filenames
 d2 =dir('*p*-s*_Segment_1.asc'); %SOME SMALL LETTER TO SEARCH FOR

 d = [d1 ; d2]; %COMBINE FILENAMES

 ylbl = cell(1,size(d,1)); %FIGURE 3 extra electrodes INITIALISE
```

```
fname_ppes = d(1).name;
data_ppes = importdata(fname_ppes);
data_ppes = -1*(data_ppes.data); %LOAD PPES DATA

%data_ppes(:,39:42) = [];

if isempty(folder_list{k,4})
channel_list = 1:size(data_ppes,2);
else
channel_list = folder_list{k,4};
end %DEFINE X LABEL OF FIGURE 3



gStim = zeros(size(data_ppes,2),size(d,1));
gSup_e = zeros(size(data_ppes,2),size(d,1));
gSup_h = zeros(size(data_ppes,2),size(d,1));
gDep_e = zeros(size(data_ppes,2),size(d,1));
gDep_h = zeros(size(data_ppes,2),size(d,1));
gFac_e = zeros(size(data_ppes,2),size(d,1));
gFac_h = zeros(size(data_ppes,2),size(d,1));
gSim_e = zeros(size(data_ppes,2),size(d,1));
gSim_h = zeros(size(data_ppes,2),size(d,1));
ghspes_pc = zeros(size(data_ppes,2),size(d,1));
gespes_pc = zeros(size(data_ppes,2),size(d,1));
gchan_notused = zeros(size(data_ppes,2),size(d,1));
gError_h = zeros(size(data_ppes,2),size(d,1));
gError_e = zeros(size(data_ppes,2),size(d,1));
gppes_h = zeros(size(data_ppes,2),size(d,1));
gspes_h = zeros(size(data_ppes,2),size(d,1));
gppes_e = zeros(size(data_ppes,2),size(d,1));
gspes_e = zeros(size(data_ppes,2),size(d,1));
gNALS = zeros(size(data_ppes,2),size(d,1));
gNALP = zeros(size(data_ppes,2),size(d,1));
```

```
gSup_e_n = zeros(size(data_ppes,2),size(d,1));
gSup_h_n = zeros(size(data_ppes,2),size(d,1));
gDep_e_n = zeros(size(data_ppes,2),size(d,1));
gDep_h_n = zeros(size(data_ppes,2),size(d,1));
gFac_e_n = zeros(size(data_ppes,2),size(d,1));
gFac_h_n = zeros(size(data_ppes,2),size(d,1));
gSim_e_n = zeros(size(data_ppes,2),size(d,1));
gSim_h_n = zeros(size(data_ppes,2),size(d,1));
ghspes_pc_n = zeros(size(data_ppes,2),size(d,1));
gespes_pc_n = zeros(size(data_ppes,2),size(d,1));
gError_h_n = zeros(size(data_ppes,2),size(d,1));
gError_e_n = zeros(size(data_ppes,2),size(d,1));
gppes_h_n = zeros(size(data_ppes,2),size(d,1));
gspes_h_n = zeros(size(data_ppes,2),size(d,1));
gppes_e_n = zeros(size(data_ppes,2),size(d,1));
gspes_e_n = zeros(size(data_ppes,2),size(d,1));

for i = 18:size(d,1)

fname_ppes = d(i).name;

%LOAD DATA (BEFORE ARTEFACT CORRECTION)
%ppes
data_ppes = importdata(fname_ppes);
data_ppes = -1*(data_ppes.data);
%data_ppes(:,39:42) = [];
%spes
SNAME = extract_filename(fname_ppes); %LOADS FUNCTION extract_filename.m
to find SPES filename
sname1=regexprep(SNAME, '_Segment_1.asc', '');

if exist(SNAME,'file') %CHECK IF SPES FILE ACTUALLY EXISTS

data_spes = importdata(SNAME);
```

```
data_spes = -1*(data_spes.data);
%data_spes(:,39:42) = [];

%LOAD DATA (AFTER ARTEFACT CORRECTION )
cd tms
data_spes_tms = importdata(SNAME);
data_spes_tms = -1*(data_spes_tms.data);
%data_spes_tms(:,39:42) = [];
data_ppes_tms = importdata(fname_ppes);
data_ppes_tms = -1*(data_ppes_tms.data);
%data_ppes_tms(:,39:42) = [];
cd ..

%NOW WE HAVE SPES,PPES before and after ARTEFACT CORRECTION




% B. ANALYSE DATA

[channel_use, m_amps,
m_amps2,m_amps3,m_indices,m_indices2,m_indices3,NALS,spes_ind,
ppes_ind,NALP] = analyse_data_v2(data_spes,data_ppes,data_spes_tms,data_ppes_tms);
%channel_use is channels that have a response (=1)
%m_amps is amplitudes of SPES response
%m_amps2 is PPES with OLD method
%m_amps3 is PPES with NEW method
%m_indices 1,2,3
%NALS noise level of SPES
%spes_ind where the SPES artefact is
%ppes_ind where the PPES (2nd pulse) artefact is
%NALP noise level of PPES
```

```
%COMPARISONS - INITIALISING


[e_ratio_pc,h_ratio_pc,Sup_h,Dep_h,Sim_h,Fac_h,Sup_e,Dep_e,Sim_e,Fac_e,Error_e,Er
ror_h,e_noresponse,h_noresponse,h_spessample,e_spessample,h_ppessample,e_ppessam
ple] =
classify_data(m_amps,m_amps2,m_indices,m_indices2,NALS,NALP,channel_use);

[e_ratio_pc_n,h_ratio_pc_n,Sup_h_n,Dep_h_n,Sim_h_n,Fac_h_n,Sup_e_n,Dep_e_n,Sim
_e_n,Fac_e_n,Error_e_n,Error_h_n,e_noresponse_n,h_noresponse_n,h_spessample_n,e_
spessample_n,h_ppessample_n,e_ppessample_n] =
classify_data(m_amps,m_amps3,m_indices,m_indices3,NALS,NALP,channel_use);

 Nchannels = 1:size(m_amps,1);
 stim = zeros(size(Dep_e,1),1); %WORK OUT THE STIMULUS CHANNELS
 [stim_ind1 stim_ind2] = name2stimuli_v3(SNAME);

 % extra electrodes in y labell
 if ((stim_ind1 == 'j') || (stim_ind1 == 'i') || ~isnumeric(stim_ind1)) && ((stim_ind2 == 'j')
|| (stim_ind2 == 'i') || ~(isnumeric(stim_ind2)))
 ylbl{i} = [stim_ind1 '_' stim_ind2];
 elseif (stim_ind1 == 'j') || (stim_ind1 == 'i') || ~isnumeric(stim_ind1)
 stim(find(channel_list == stim_ind2)) = 50;
 ylbl{i} = stim_ind1;

 elseif (stim_ind2 == 'j') || (stim_ind2 == 'i') || ~(isnumeric(stim_ind2))
 stim(find(channel_list == stim_ind1)) = 100;
 ylbl{i} = stim_ind2;
 else
 stim(find(channel_list == stim_ind1)) = 100;
 stim(find(channel_list == stim_ind2)) = 50;
 end

 if isempty(find(channel_list == stim_ind1)) | isempty(find(channel_list == stim_ind2))
```

```
 if ~(((stim_ind1 == 'j') || (stim_ind1 == 'i') || ~isnumeric(stim_ind1)) || ((stim_ind2 == 'j')
|| (stim_ind2 == 'i') || ~(isnumeric(stim_ind2))))

 H = warndlg('This file contains stimulation channels greater than the number of recorded
ones',SNAME);
 uiwait(H);
 end

 end

 save_name = strcat(char(folder_list(k)),'_Figure1_', SNAME(1:end-4));

 figure('Name',save_name,'NumberTitle','off') %CREATE FIRST FIGURE
 plot_spes_ppes(data_spes_tms,
data_ppes_tms,spes_ind,ppes_ind,m_indices,m_indices2,m_indices3);
 screen_size = get(0, 'ScreenSize');5
 set(gcf, 'Position', [screen_size(3)/4 screen_size(4)/4 screen_size(3)*(2/3)
screen_size(4)*(3/4) ] );
 %m_amps = max1 max2 min1 min2
 cd Figure1_spes_ppes
 saveas(gcf,[save_name '.fig'] ,'fig'); %SAVE FIGURES
 saveas(gcf,[save_name '.jpeg'],'jpeg');
 close
 cd ..

 %PLOT ALL DATA - HISTOGRAM
 save_name2 = strcat(char(folder_list(k)),'_Figure2_', SNAME(1:end-4));
 figure('Name',save_name2,'NumberTitle','off')

plot_hists(e_ratio_pc,h_ratio_pc,Sup_e,Dep_e,Fac_e,Sim_e,Sup_h,Dep_h,Fac_h,Sim_h,
Error_e,Error_h,channel_use,h_noresponse,e_noresponse,Nchannels,stim)
 cd Figure2_histogram_old
 saveas(gcf,[save_name2 '.fig'] ,'fig');
```

```
saveas(gcf,[save_name2 '.jpeg'],'jpeg');
close
cd ..

save_name3 = strcat(char(folder_list(k)),'_Figure3_', SNAME(1:end-4));
figure('Name',save_name3,'NumberTitle','off')

plot_hists(e_ratio_pc_n,h_ratio_pc_n,Sup_e_n,Dep_e_n,Fac_e_n,Sim_e_n,Sup_h_n,Dep
_h_n,Fac_h_n,Sim_h_n,Error_e_n,Error_h_n,channel_use,h_noresponse_n,e_noresponse
_n,Nchannels,stim)
cd Figure2_histogram_new
saveas(gcf,[save_name2 '.fig'] ,'fig');
saveas(gcf,[save_name2 '.jpeg'],'jpeg');
close
cd ..


USES new analysis method
gStim(:,i) = stim;
gSup_e(:,i) = Sup_e;
gSup_h(:,i) = Sup_h;
gDep_e(:,i) = Dep_e;
gDep_h(:,i) = Dep_h;
gFac_e(:,i) = Fac_e;
gFac_h(:,i) = Fac_h;
gSim_e(:,i) = Sim_e;
gSim_h(:,i) = Sim_h;
ghspes_pc(:,i) = e_ratio_pc;
gespes_pc(:,i) = h_ratio_pc;
gchan_notused(:,i) = ((1-channel_use) + e_noresponse)*(-50);
gError_h(:,i) = Error_h;
gError_e(:,i) = Error_e;
gppes_h(:,i) = h_ppessample;
gspes_h(:,i) = h_spessample;
```

```
gppes_e(:,i) = e_ppessample;
gspes_e(:,i) = e_spessample;
gNALS(:,i) = NALS;
gNALP(:,i) = NALP;

gSup_e_n(:,i) = Sup_e_n;
gSup_h_n(:,i) = Sup_h_n;
gDep_e_n(:,i) = Dep_e_n;
gDep_h_n(:,i) = Dep_h_n;
gFac_e_n(:,i) = Fac_e_n;
gFac_h_n(:,i) = Fac_h_n;
gSim_e_n(:,i) = Sim_e_n;
gSim_h_n(:,i) = Sim_h_n;
ghspes_pc_n(:,i) = e_ratio_pc_n;
gespes_pc_n(:,i) = h_ratio_pc_n;
gchan_notused(:,i) = ((1-channel_use) + e_noresponse_n)*(-50);
gError_h_n(:,i) = Error_h_n;
gError_e_n(:,i) = Error_e_n;
gppes_h_n(:,i) = h_ppessample_n;
gspes_h_n(:,i) = h_spessample_n;
gppes_e_n(:,i) = e_ppessample_n;
gspes_e_n(:,i) = e_spessample_n;



abs_h = abs(h_spessample-h_ppessample);
abs_e = abs(e_spessample-e_ppessample);
abs_h_n = abs(h_spessample_n-h_ppessample_n);
abs_e_n = abs(e_spessample_n-e_ppessample_n);

data = [Nchannels' m_amps m_indices m_amps2 m_indices2 m_amps3 m_indices3
e_spessample h_spessample e_ppessample h_ppessample e_ratio_pc h_ratio_pc
e_spessample_n h_spessample_n e_ppessample_n h_ppessample_n e_ratio_pc_n
h_ratio_pc_n NALS NALP abs_e abs_h abs_e_n abs_h_n]';
```

```
data_header = {'Channel' 'max1' 'max2' 'min1' 'min2' 'max1_ind' 'max2_ind' 'min1_ind'
'min2_ind' 'pmax1' 'pmax2' 'pmin1' 'pmin2' 'pmax1_ind' 'pmax2_ind' 'pmin1_ind'
'pmin2_ind' 'pmax1_n' 'pmax2_n' 'pmin1_n' 'pmin2_n' 'pmax1_ind_n' 'pmax2_ind_n'
'pmin1_ind_n' 'pmin2_ind_n' 'e_spes' 'h_spes' 'e_ppes' 'h_ppes' 'e_ratio' 'h_ratio'
'e_spes_n' 'h_spes_n' 'e_ppes_n' 'h_ppes_n' 'e_ratio_n' 'h_ratio_n' 'NALS' 'NALP' 'Abs_e'
'Abs_h' 'Abs_e_n' 'Abs_h_n'}';
data_to_xls = [data_header num2cell(data)];

data1 = [Nchannels' m_amps m_indices]';
data1_header = {'Channel' 'max1' 'max2' 'min1' 'min2' 'max1_ind' 'max2_ind' 'min1_ind'
'min2_ind'}';
data1_to_xls = [data1_header num2cell(data1)];

data2 = [m_amps2 m_indices2]';
data2_header = {'pmax1' 'pmax2' 'pmin1' 'pmin2' 'pmax1_ind' 'pmax2_ind' 'pmin1_ind'
'pmin2_ind'}';
data2_to_xls = [data2_header num2cell(data2)];

data3 = [m_amps3 m_indices3]';
data3_header = {'pmax1_n' 'pmax2_n' 'pmin1_n' 'pmin2_n' 'pmax1_ind_n'
'pmax2_ind_n' 'pmin1_ind_n' 'pmin2_ind_n'}';
data3_to_xls = [data3_header num2cell(data3)];

data4 = [e_spessample h_spessample e_ppessample h_ppessample e_ratio_pc
h_ratio_pc]';
data4_header = {'e_spes' 'h_spes' 'e_ppes' 'h_ppes' 'e_ratio' 'h_ratio'}';
data4_to_xls = [data4_header num2cell(data4)];

data5 = [e_spessample_n h_spessample_n e_ppessample_n h_ppessample_n
e_ratio_pc_n h_ratio_pc_n]';
data5_header = {'e_spes_n' 'h_spes_n' 'e_ppes_n' 'h_ppes_n' 'e_ratio_n' 'h_ratio_n'}';
data5_to_xls = [data5_header num2cell(data5)];

data6 = [NALS NALP abs_e abs_h abs_e_n abs_h_n]';
```

```
data6_header = {'NALS' 'NALP' 'Abs_e' 'Abs_h' 'Abs_e_n' 'Abs_h_n'}';
data6_to_xls = [data6_header num2cell(data6)];


Excel = actxserver('Excel.Application'); %connects with excel
File= [pwd '\' char(folder_list(k)) '.xls'];

if ~exist(File,'file')
ExcelWorkbook = Excel.workbooks.Add;
ExcelWorkbook.SaveAs(File,1);
ExcelWorkbook.Close(false);

invoke(Excel.Workbooks,'Open',File);

xlswrite1(File,data1_to_xls,sname1,'A1');
xlswrite1(File,data2_to_xls,sname1,'A11');
xlswrite1(File,data3_to_xls,sname1,'A20');
xlswrite1(File,data4_to_xls,sname1,'A29');
xlswrite1(File,data5_to_xls,sname1,'A37');
xlswrite1(File,data6_to_xls,sname1,'A44');


worksheets = Excel.sheets;

sheetIdx = 1;
sheetIdx2 = 1;
numSheets = 3;
while sheetIdx2 <= numSheets
sheetName = worksheets.Item(sheetIdx).Name(1:end-1);
if ~isempty(strmatch(sheetName,'Sheet'))
worksheets.Item(sheetIdx).Delete;
else
Move to the next sheet
sheetIdx = sheetIdx + 1;
```

```
end
sheetIdx2 = sheetIdx2 + 1; % prevent endless loop...
end

invoke(Excel.ActiveWorkbook,'Save');
Excel.Workbooks.Close;

else
invoke(Excel.Workbooks,'Open',File);
xlswrite1(File,data_to_xls,sname1,'A1');
xlswrite1(File,data1_to_xls,sname1,'A1');
xlswrite1(File,data2_to_xls,sname1,'A11');
xlswrite1(File,data3_to_xls,sname1,'A20');
xlswrite1(File,data4_to_xls,sname1,'A29');
xlswrite1(File,data5_to_xls,sname1,'A37');
xlswrite1(File,data6_to_xls,sname1,'A44');

invoke(Excel.ActiveWorkbook,'Save');
Excel.Workbooks.Close
end
end
end

%END OF MAIN FOR LOOP
Excel.Quit
Excel.delete
clear Excel
%
%save([char(folder_list(k))
'.mat'],'gStim','gSim_h','gSim_e','gFac_e','gFac_h','gDep_e','gDep_h','gSup_h','gSup_e',
'ghspes_pc',
'gespes_pc','gError_e','gError_h','gchan_notused','gppes_h','gspes_h','gppes_e','gspes_e','g
NALS','gNALP','gStim_n','gSim_h_n','gSim_e_n','gFac_e_n','gFac_h_n','gDep_e_n','gDe
p_h_n','gSup_h_n','gSup_e_n', 'ghspes_pc_n',
```

```
'gespes_pc_n','gError_e_n','gError_h_n','gppes_h_n','gspes_h_n','gppes_e_n','gspes_e_n');

% cd('/Users/ae301/Desktop/Projects 2011/EEG Data - Kings/ASCII/RESULTS/ASCII
Intracranial Figure3')
% mkdir(['ASCII ' char(folder_list(k))]);
% cd(['ASCII ' char(folder_list(k))]);

mkdir Figure3_final_plot_new
mkdir Figure3_final_plot_old

fig_title = folder_list{k,2};
fig_bold = folder_list{k,3};

cd Figure3_final_plot_old
plot_3d_v4(gStim,gDep_e,gFac_e,gSup_e,gSim_e,gNALS,gDep_h,gFac_h,gSup_h,gSim
_h,gspes_e,gspes_h,ylbl,channel_list,fig_title,fig_bold);
cd ..

cd Figure3_final_plot_new
plot_3d_v4(gStim,gDep_e_n,gFac_e_n,gSup_e_n,gSim_e_n,gNALS,gDep_h_n,gFac_h_
n,gSup_h_n,gSim_h_n,gspes_e_n,gspes_h_n,ylbl,channel_list,fig_title,fig_bold);

cd('/Users/ae301/Desktop/Projects 2011/EEG Data - Kings/ASCII/RESULTS/ASCII
FILES');


close all


end



function [channel_use, m_amps,
```

```
m_amps2,m_amps3,m_indices,m_indices2,m_indices3,NALS,spes_ind,
ppes_ind,NALP] = analyse_data_v2(data_spes,data_ppes,data_spes_tms,data_ppes_tms)

N = size(data_spes,2); %NUMBER OF CHANNELS

if size(data_ppes,2) ~= N %CHECK IS PPES AND SPES HAVE SAME NUMBER OF
CHANNELS
 errordlg('Error: ppes and spes data have different channel counts!');
else

 %define some variables (makes computation faster)
 m_indices = zeros(N,4);
 m_amps = zeros(N,4);
 m_indices2 = zeros(N,4);
 m_amps2 = zeros(N,4);
 m_indices3 = zeros(N,4);
 m_amps3 = zeros(N,4);

 spes_ind = zeros(N,1);
 ppes_ind = zeros(N,1);

 channel_use = zeros(N,1); %this will be 1 if channel is usable, 0 otherwise

 %DEFINE SAMPLING FREQUENCY
 if (length(data_spes) == 600) | (length(data_spes)==800);
 Fs = 200;
 %x=1:600 | x=1:800;
 elseif (length(data_spes) == 768) | (length(data_spes)==1024);
 Fs = 256;
 %x=1:768 | x=1:1024;
 elseif (length(data_spes) == 1536) | (length(data_spes)==2048);
 Fs = 512;
 %x=1:1536 | x=1:2048;
 else (length(data_spes) == 3072) | (length(data_spes)==4096);
```

```
Fs=1024;
%x=1:3072 | x=1:4096;
end

ms_look = 50; %number of ms to look around a maxima/minima in ppes - 25ms either side
samp_look = round((ms_look*1e-3)*Fs); %HOW MANY SAMPLE POINTS IS ms_look

if mod(samp_look,2) ~= 0 %IF samp_look is not even add 1 to it
samp_look = samp_look +1;
end

dur = 0.5; %500ms window
dur_samps = dur*Fs;

%WE NOW KNOW THE WINDOW LENGTH (500ms) and in OLD analysis the smaller
%window (50ms)

%check max/min of each channel
for i = 1:N %GOING THROUGH EACH CHANNEL

% Noise amplitude level for SPES (NALS)
if Fs==200;
bsls=data_spes(4:204, i);
bsls_ppes_tms = data_ppes_tms(4:204, i);
bsls_ppes = data_ppes(4:204, i);
elseif Fs==256;
bsls=data_spes(5:261, i);
bsls_ppes_tms = data_ppes_tms(5:261, i);
bsls_ppes = data_ppes(5:261, i);
elseif Fs==512;
bsls=data_spes(10:522, i);
```

```
bsls_ppes_tms = data_ppes_tms(10:522, i);
bsls_ppes = data_ppes(10:522, i);
else
bsls=data_spes(20:1044, i);
bsls_ppes_tms = data_ppes_tms(20:1044, i);
bsls_ppes = data_ppes(20:1044, i);

end

data_spes(:,i) = data_spes(:,i) - mean(bsls); %REMOVES ANY LARGE OFFSETS IN
DATA (SPES)

NALS(i,:) = 2*std(bsls);
NALP(i,:) = 2*std(bsls_ppes_tms);
NALP_ntms(i,:) = 2*std(bsls_ppes);

m = max(abs(data_spes(:,i))); %THE MAXIMUM POINT IN THE SIGNAL
(ARTEFACT)
d = m - NALS(i,:);

if d < 300
channel_use(i,1) = 0;
pes_ind_area = floor(size(data_ppes,1)/4):floor(size(data_ppes,1)*(3/4));
[m, spes_ind(i,1)] = max(abs(data_spes(pes_ind_area,i))); %INFORMATION FOR
OTHER FUNCTIONS
[m, ppes_ind(i,1)] = max(abs(data_ppes(pes_ind_area,i))); %INFORMATION FOR
OTHER FUNCTIONS

spes_ind(i,1) = spes_ind(i,1) + pes_ind_area(1);
ppes_ind(i,1) = ppes_ind(i,1) + pes_ind_area(1);
else

[m_indices(i,:), m_amps(i,:),spes_ind(i,1),ind1] =
maxmin_finder_v2(data_spes(:,i),data_spes_tms(:,i),Fs); %LOOKING FOR MAX/MIN
```

```
IN THE RESPONSE
 %mamps = (['max1 max2 min1 min2']);

 Num_Zeros = length(find(m_amps(i,:) == 0)); %HOW MANY MAXIMA/MINIMA
ARE THERE IN THE RESPONSE? HOW MANY ZEROS?

 %define if channel is usable
 lgc = any(m_indices(i,1:2) ~=0) & any(m_indices(i,3:4) ~=0); %WERE THERE
MAX/MIN T THE EDGE OF 500ms WINDOW

 if (Num_Zeros <=2) & lgc

 channel_use(i,1) = 1; %CHANNEL USABLE
 data_ppes(:,i) = data_ppes(:,i) - mean(bsls_ppes); %REMOVE OFFSET FROM PPES
JUST IN CASE

 m = max(abs(data_ppes(:,i))); %FIND THE PPES ARTEFACT


 %OLD ANALYSIS

 [area_to_check,ppes_ind(i,:)] =
ppes_cmpr(data_ppes(:,i),samp_look,m_indices(i,:),Num_Zeros,dur_samps,Fs);
 %DEFINES THE SAMPLE POINTS IN PPES SIGNAL TO LOOK FOR
 %RESPONSES IN SMALLER WINDOWS - OLD METHOD

 ind_zeros = (m_indices(i,:) == 0);

 d = m - NALP_ntms(i,:);

 if d > 300
 for j = 1:size(area_to_check,1)
 if ind_zeros(j) == 0 %IS WINDOW USEABLE?
 areas = area_to_check(j,:);
```

```
 areas(areas==0) = []; %REMOVE ANY ZEROS BECAUSE CAN'T USE WHOLE
50ms
 if (j == 1)||(j==2)
 [m_amps2(i,j),m_indices2(i,j)] = max(data_ppes_tms(areas,i)); %LOOK FOR
MAXIMA IN SMALL WINDOW
 else
 [m_amps2(i,j),m_indices2(i,j)] = min(data_ppes_tms(areas,i)); %LOOK FOR MINIMA
IN SMALL WINDOW
 end
 m_indices2(i,j) = m_indices2(i,j) + areas(1)-1;%DEFINES THE ACTUAL INDEX OF
THE MAXIMA/MINIMA
 end



 end

% ADDED TO CHECK IF RESPONSE IS LESS THAN NOISE FLOOR,
% IF SO IGNORE IT!
% ind_lNALP = find(abs(m_amps2(i,:)) < NALP(i,:));
% if ~isempty(ind_lNALP)
% m_amps2(i,ind_lNALP) = 0; m_indices2(i,ind_lNALP) = 0;
% end

 %NEW ANALYSIS
 [m_indices3(i,:), m_amps3(i,:),~,ind2] =
maxmin_finder_v2(data_ppes(:,i),data_ppes_tms(:,i),Fs);
 else
 m_indices3(i,:) = zeros(1,4);
 m_indices2(i,:) = zeros(1,4);
 m_amps2(i,:) = zeros(1,4);
 m_amps3(i,:) = zeros(1,4);

 end
```

```
else
[m, ppes_ind(i,1)] = max(abs(data_ppes(:,i))); %FOR OTHER FUNCTIONS
channel_use(i,1) = 0; %CHANNEL CAN'T BE USED
end

m_indices(i,m_indices(i,:) ~= 0) = m_indices(i,m_indices(i,:) ~=0) + ind1;
if exist('ind2')
m_indices3(i,m_indices3(i,:)~=0) = m_indices3(i,m_indices3(i,:)~=0) + ind2;
end
end

%define areas of ppes to look for comparable maxinma/minima

%compare

%store results


end


end



function [areas,ppes_ind] =
ppes_cmpr(x,samp_look,m_ind,Num_Zeros,dur_samps,Fs,ppes_ind)
%% PPES DEFINE THE AREAS FOR OLD METHOD

if Fs==200;
 bsls=x(4:204);
elseif Fs==256;
 bsls=x(5:261);
elseif Fs==512;
```

```
 bsls=x(10:522);
else
 bsls=x(20:1044);
end

x = x-mean(bsls);

if nargin == 6 %IF NUMBER OF INPUTS < 7
 pes_ind_area = floor(size(x,1)/4):floor(size(x,1)*(3/4)); %LIMITS WHERE TO LOOK FOR PPES ARTEFACT

 [m, ppes_ind] = max(abs(x(pes_ind_area))); %the largest point in the signal (your ppes artifact)

 ppes_ind = ppes_ind+pes_ind_area(1); %ACTUAL INDEX OF PPES RESPONSE IN SIGNAL

 m = x(ppes_ind); %since we used abs() we need to know if the spes pulse is negative or positive so we set m = to the value of the spes pulse

 if m < 0 %if spes pulse is negative
 ind = find(x(ppes_ind:end) > m/100); %then find the point after the ppes pulse that is close to ppes amplitude (m) /100
 else
 ind = find(x(ppes_ind:end) < m/100); %otherwise find the point less than m/100
 end


 if isempty(ind) | (ind > 15) %make sure it's not empty
 ind = 5;
 else
 ind = ind(1)+2; %since the last two statements will give us lots of values we only want the first, i.e. the next value after the spes that is close to 0 and we add 2 just to push it a bit further
```

```
 end
else
 ind = 0; % if we have given spes_ind then we don't need to define it
end

ind_investigate = ppes_ind+ind:(ppes_ind + dur_samps-1); %so this is the final area in x,
e.g. from the 1:1536 data where to look for a response

Area_size = min([floor(dur_samps/Num_Zeros) samp_look]);
% max1 max2 min1 min2

areas = zeros(size(m_ind,2),Area_size);

ind_zeros = (m_ind == 0);

for i = 1:size(m_ind,2)
 if ind_zeros(i) == 0
 areas(i,:) = ind_investigate(1) + [(m_ind(i)- floor(Area_size/2)):(m_ind(i) +
floor(Area_size/2) -1)];

 N_test = m_ind(i) - floor(Area_size/2); %MAKE SURE WHERE WE LOOK IS IN
500ms WINDOW
 if N_test < 0;
 areas(i,1:abs(N_test)) = 0;
 end

 N_test2 = m_ind(i) + floor(Area_size/2);
 if N_test2 < 0
 areas(i,abs(N_test2):end) = 0;
 end

 end
end
```

```
function [m_indices, m_amps,sind,ind] = maxmin_finder_v2(x,xtms,Fs)
%% FUNCTION [m_indices m_amps] = maxmin_finder(x,spes_ind)
% Created by Amir Eftekhar on 1/11/2010
% Version 1.1
%
% Inputs: x = the signal of interest (one channel 1xN in size)
% spes_ind = where the spes artifact is in x
% Outputs: m_indices = indices of max1 max2 min1 min2 - 4 possible
% maxima/minima
% m_amps = the corresponding amplitudes of these maxima/minima
%
% Example (spes, no TMS) => [m_indices m_amps] = maxmin_finder(data(:,13)');
% Example (spes, TMS, 512Hz, 3sec file) => [m_indices m_amps] =
maxmin_finder(data(:,13)',768);
%
%
%


% Invert the signal as it seem to be the opposite polarity
%x = -1*x;

% If we don't tell the funtion what spes_ind (where the spes pulse is in
% the file) then we need to calculate it
if Fs==200;
 bsls=x(4:204);
elseif Fs==256;
 bsls=x(5:261);
elseif Fs==512;
 bsls=x(10:522);
else
 bsls=x(20:1044);
```

```
end

x = x-mean(bsls);

pes_ind_area = floor(size(x,1)/4):floor(size(x,1)*(3/4));
[m, spes_ind] = max(abs(x(pes_ind_area))); %the largest point in the signal (your spes artifact)
spes_ind = spes_ind+pes_ind_area(1);
m = x(spes_ind); %since we used abs() we need to know if the spes pulse is negative or positive so we set m = to the value of the spes pulse

if m < 0 %if spes pulse is negative
 ind = find(x(spes_ind:end) > m/100); %then find the point after the spes pulse that is close to spes amplitude (m) /100
else
 ind = find(x(spes_ind:end) < m/100); %otherwise find the point less than m/100
end

if isempty(ind) | (ind > 15) %make sure it's not empty
 ind = 5;
else
 ind = ind(1)+2; %since the last two statements will give us lots of values we only want the first, i.e. the next value after the spes that is close to 0 and we add 2 just to push it a bit further
end


dur = 0.5; %s %we want to look at 0.5 seconds after spes for a response
dur_sample = dur*Fs; %this corresponds to the duration (in seconds) multiplied by the sampling frequency

ind_investigate = spes_ind+ind:min([size(xtms,1) (spes_ind + dur_sample-1)]); %so this is the final area in x, e.g. from the 1:1536 data where to look for a response
```

```
if ~isempty(ind_investigate)
 [max1, max_ind1] = max(xtms(ind_investigate)); %find the maxima in this area (max1 is the amplitude and max_ind1 the index)
 [min1, min_ind1] = min(xtms(ind_investigate)); %similar for minima
 max_ind1 = max_ind1-1; %need to just correct the index because of
 min_ind1= min_ind1-1; %same for the min

 %Now we have potentially 2 points a maxima and a minima, now we need to
 %find the others...
 if max_ind1 > min_ind1 %if the minima came first
 [min2, min_ind2] = min(xtms(ind_investigate(max([max_ind1 1]):end))); %then in the data after the maxima see if there is another minima
 min_ind2 = min_ind2+max_ind1-2; %correct the index a bit
 if (abs(min_ind2 - max_ind1-1) < Fs*0.04) & (min_ind1 > 2) %is this new minima very close to the previous maxima? (closer than 40ms)
 [max2, max_ind2] = max(xtms(ind_investigate(1:min_ind1))); %yes - ok look for a maxmima before the 1 st minima found
 max_ind2 = max_ind2-1; %offset correct
 min_ind2 = 0; %since the minima we found was too close we remove it
 min2 = 0; %and the amplitude of it
 else
 max2 = 0; %if the minima was ok (outside 40ms) then we don't need to look for another maxima
 max_ind2 = 0;
 end
 else
 %exactly the same argument for there being the maxima first followed by a minima (from the original 2 points found)
 [max2, max_ind2] = max(xtms(ind_investigate(max([min_ind1 1]):end)));
 max_ind2 = max_ind2+min_ind1-2;
 if (max_ind2 - min_ind1-1 < Fs*0.04) & (max_ind1 > 2)
 [min2, min_ind2] = min(xtms(ind_investigate(1:max_ind1)));
 min_ind2 = min_ind2-1;
 max2 = 0;
```

```
max_ind2 = 0;

else
min2 = 0;
min_ind2 = 0;
end

end

%Ok, now we have all the points we want to find, lets put them in our
%outputs
%disp(['max1 max2 min1 min2']);
m_indices = [max_ind1 max_ind2 min_ind1 min_ind2];
m_amps = [max1 max2 min1 min2];

%Here we just check that the maxima/minima indices are not at the end
%or beginning of the window we were investigating, otherwise they are
%not true minima/maxima
if (max_ind1 == 1) | (max_ind1 == length(ind_investigate)-1)
m_indices(1) = 0;
m_amps(1) = 0;
end
if (max_ind2 == 1) | (max_ind2 == length(ind_investigate)-1)
m_indices(2) = 0;
m_amps(2) = 0;
end
if (min_ind1 == 1) | (min_ind1 == length(ind_investigate)-1)
m_indices(3) = 0;
m_amps(3) = 0;
end
if (min_ind2 == 1) | (min_ind2 == length(ind_investigate)-1)
m_indices(4) = 0;
m_amps(4) = 0;
end
```

```
 sind = spes_ind;
 ind = ind_investigate(1);
else
 ind = spes_ind;
 sind = spes_ind;
 m_indices = [0 0 0 0];
 m_amps = [0 0 0 0];

end


function [] =
plot_spes_ppes(data_spes,data_ppes,spes_ind,ppes_ind,mind1,mind2,mind3)

N = size(data_spes,2);
S = 600;
k = S:S:N*S;

ax(1) = subplot(121);
hold all
n1 = floor(size(data_spes,1)/2);
for i = 1:N



 plot(k(i) + data_spes(:,i),'b')



 if (spes_ind(i,:) < (n1*(3/2))) && (spes_ind(i,:) > (n1/2))

 ind = spes_ind(i,:)-5:spes_ind(i,:)+5;
```

```
if all(ind > 0)

plot(ind,k(i) + data_spes(ind,i),'-r');
end

ind = sort(mind1(i,find(mind1(i,:) ~=0)));

if ~isempty(ind)
plot(ind,k(i) + data_spes(ind,i),'xk','MarkerSize',10);
end
end

end

% set(gca,'Box','off')
% set(gca,'XTick',[])
% set(gca,'YTick',[])
Pos = get(gca,'OuterPosition');
Pos(2) = 0.05;
Pos(3) = Pos(3)+ 0.02;
Pos(4) = Pos(4) - 0.25;
set(gca,'Position',Pos)
xlim([0 size(data_spes,1)]);
ylim([0 (S*N)+200]);
set(gca,'YTick',[0 k]);
set(gca,'YTickLabel',num2cell(0:N))
Xlabel('SPES');

ax(2) = subplot(122);
hold all

for i = 1:N

plot(k(i) + data_ppes(:,i),'b')
```

```
if (ppes_ind(i,:) < (n1*(3/2))) && (ppes_ind(i,:) > (n1/2))

ind = ppes_ind(i,:)-5:ppes_ind(i,:)+5;

if all(ind > 0)
plot(ind,k(i) + data_ppes(ind,i),'-r');
% if ind(1)-107 > 0
% plot(ind-107,k(i) + data_ppes(ind-107,i),'-r');
% end
end


ind = sort(mind2(i,find(mind2(i,:) ~=0)));
plot(ind,k(i) + data_ppes(ind,i),'xk','MarkerSize',10);

ind = sort(mind3(i,find(mind3(i,:) ~=0)));
plot(ind,k(i) + data_ppes(ind,i),'.k','MarkerSize',6);
end
end
xlim([0 size(data_ppes,1)]);
ylim([0 (S*N)+200]);
set(gca,'YTick',[0 k]);
set(gca,'YTickLabel',num2cell(0:N))
% set(gca,'Box','off')
% set(gca,'XTick',[])
% set(gca,'YTick',[])
Pos = get(gca,'OuterPosition');
Pos(2) = 0.05;
Pos(3) = Pos(3)+ 0.02;
Pos(4) = Pos(4) - 0.25;
set(gca,'Position',Pos)
```

```
Xlabel('PPES');

linkaxes(ax,'y');


function [pos neg] = name2stimuli_v3(fname)

temp1 = regexp(fname,'_s(\d+)\w_','ignorecase','match');
temp2 = regexp(fname,'_s\w(\d+)_','ignorecase','match');
temp3 = regexp(fname,'_s\w\w_','ignorecase','match');

if isempty(temp1)
 temp = temp2;
else isempty(temp2)
 temp = temp1;
end

if (isempty(temp1)&& isempty(temp2))
 Mstr = regexprep(temp3, '_s','','ignorecase');
 Mstr = Mstr{1}(1:end-1);
 pos = Mstr(1);
 neg = Mstr(2);

else


 Mstr = regexprep(temp, '_s','','ignorecase');


 x =0;

 if size(Mstr,2) > 1
```

```
for i = 1:size(Mstr,2)
xn = length(Mstr{i});
if xn > x
m = i;
x = xn;
end

end
else
m =1;
end


Mstr = Mstr{m};
Mstr = Mstr(1:end-1);

switch size(Mstr,2)
case 2
pos = str2double(Mstr(1));
neg = str2double(Mstr(2));
if isnan(pos)
pos = Mstr(1);
end
if isnan(neg)
neg = Mstr(2);
end
case 3
pos1 = str2double(Mstr(1));
pos2 = str2double(Mstr(1:2));
neg1 = str2double(Mstr(2:3));
neg2 = str2double(Mstr(3));

if isnan(pos1) || (Mstr(1) == 'j') || (Mstr(1) == 'i')
pos = Mstr(1);
```

```
 neg = str2double(Mstr(2:3));
 elseif isnan(neg2) || (Mstr(3) == 'j') || (Mstr(3) == 'i')
 pos = str2double(Mstr(1:2));
 neg = Mstr(3);
 else

 p1_diff = abs(pos1 -neg1);
 p2_diff = abs(pos2 -neg2);

 if (p1_diff < p2_diff) && ((pos1>=10) || (neg1>=10))
 pos = pos1; neg = neg1;
 else
 pos = pos2; neg = neg2;
 end
 end
 case 4
 pos = str2double(Mstr(1:2));
 neg = str2double(Mstr(3:4));
 end



 end
function [pos neg] = name2stimuli_v3(fname)

temp1 = regexp(fname,'_s(\d+)\w_','ignorecase','match');
temp2 = regexp(fname,'_s\w(\d+)_','ignorecase','match');
temp3 = regexp(fname,'_s\w\w_','ignorecase','match');

if isempty(temp1)
 temp = temp2;
else isempty(temp2)
 temp = temp1;
end
```

```
if (isempty(temp1)&& isempty(temp2))
 Mstr = regexprep(temp3, '_s','','ignorecase');
 Mstr = Mstr{1}(1:end-1);
 pos = Mstr(1);
 neg = Mstr(2);

else


 Mstr = regexprep(temp, '_s','','ignorecase');


 x =0;

 if size(Mstr,2) > 1

 for i = 1:size(Mstr,2)
 xn = length(Mstr{i});
 if xn > x
 m = i;
 x = xn;
 end

 end
 else
 m =1;
 end


 Mstr = Mstr{m};
 Mstr = Mstr(1:end-1);

 switch size(Mstr,2)
 case 2
```

```
pos = str2double(Mstr(1));
neg = str2double(Mstr(2));
if isnan(pos)
pos = Mstr(1);
end
if isnan(neg)
neg = Mstr(2);
end
case 3
pos1 = str2double(Mstr(1));
pos2 = str2double(Mstr(1:2));
neg1 = str2double(Mstr(2:3));
neg2 = str2double(Mstr(3));

if isnan(pos1) || (Mstr(1) == 'j') || (Mstr(1) == 'i')
pos = Mstr(1);
neg = str2double(Mstr(2:3));
elseif isnan(neg2) || (Mstr(3) == 'j') || (Mstr(3) == 'i')
pos = str2double(Mstr(1:2));
neg = Mstr(3);
else

p1_diff = abs(pos1 -neg1);
p2_diff = abs(pos2 -neg2);

if (p1_diff < p2_diff) && ((pos1>=10) || (neg1>=10))
pos = pos1; neg = neg1;
else
pos = pos2; neg = neg2;
end
end
case 4
pos = str2double(Mstr(1:2));
neg = str2double(Mstr(3:4));
```

```
 end



end
```

```
function [] =
plot_3d_v4(gStim,gDep_e,gFac_e,gSup_e,gSim_e,gNALS,gDep_h,gFac_h,gSup_h,gSim
_h,gspes_e,gspes_h,ylbl,ch_list,fig_title,fig_bold)

mx_channels_e = zeros(size(gStim));
mx_channels_e(gDep_e == 1) = 6;
mx_channels_e(gFac_e == 1) = 3;
mx_channels_e(gSup_e == 1) = 4;
mx_channels_e(gSim_e == 1) = 5;
mx_channels_e(gStim == 50) = 2;
mx_channels_e(gStim == 100) = 1;

mx_channels_e_new = zeros(size(gStim));
cat_NALS_e = struct('mx',false(size(gStim)));
cat_NALS_h = struct('mx',false(size(gStim)));

mx_channels_h = zeros(size(gStim));
mx_channels_h(gDep_h == 1) = 6;
mx_channels_h(gFac_h == 1) = 3;
mx_channels_h(gSup_h == 1) = 4;
mx_channels_h(gSim_h == 1) = 5;
```

```
mx_channels_h(gStim == 50) = 2;
mx_channels_h(gStim == 100) = 1;
mx_channels_h_new = zeros(size(gStim));
ylbl_new = cell(1,size(gStim,2));


k =1;
kNALS = 2:2:10;
i = 1;
gind_p = [];

while (k <= size(gStim,2))

 ind_p = find(mx_channels_e(i,:) == 1);
 ind_n = [];
 if ~isempty(ind_p)


 for j = 1:length(ind_p)
 n = find(mx_channels_e(:,ind_p(j))==2);
 if ~isempty(n)
 ind_n(j) = n;
 else
 ind_n(j) = j;
 end

 end


 [~,I] = sort(ind_n);

 for j = 1:length(ind_p)
 mx_channels_e_new(:,k) = mx_channels_e(:,ind_p(I(j)));
 mx_channels_h_new(:,k) = mx_channels_h(:,ind_p(I(j)));
```

```
ylbl_new{k} = ylbl{ind_p(I(j))};

for n = 1:5
cat_NALS_e(n).mx(:,k) = (gspes_e(:,ind_p(I(j))) - kNALS(n)*gNALS(:,ind_p(I(j)))) >=
0;
cat_NALS_h(n).mx(:,k) = (gspes_h(:,ind_p(I(j))) - kNALS(n)*gNALS(:,ind_p(I(j)))) >=
0;
end

k = k + 1;

end

gind_p = [gind_p ind_p];
end

i = i+1;
if i > size(gStim,1)
l = 1:size(gStim,2);



l(gind_p) = [];

for j = 1:length(l)
mx_channels_e_new(:,k) = mx_channels_e(:,l(j));
mx_channels_h_new(:,k) = mx_channels_h(:,l(j));
ylbl_new{k} = ylbl{l(j)};

for n = 1:5
cat_NALS_e(n).mx(:,k) = (gspes_e(:,l(j)) - kNALS(n)*gNALS(:,l(j))) >= 0;
cat_NALS_h(n).mx(:,k) = (gspes_h(:,l(j)) - kNALS(n)*gNALS(:,l(j))) >= 0;
end

k = k + 1;
```

```
 end


 end


end


titles = {'spessample_2std',
'spessample_4std','spessample_6std','spessample_8std','spessample_10std'};

w=1;

for i = 1:5
 figure('Name',[char(titles{i}) '_early']);
 screen_size = get(0, 'ScreenSize');
 set(gcf, 'Position', [screen_size(3)/4 screen_size(4)/4 screen_size(3)*(2/3)
screen_size(4)*(3/4) ] );

 Font_size = 6;

 %subplot(1,2,1)
 bar3((3*(mx_channels_e_new==6).*cat_NALS_e(i).mx)',w,'y')
 hold on
 bar3((3*(mx_channels_e_new==4).*cat_NALS_e(i).mx)',w,'r')
 bar3((3*(mx_channels_e_new==3).*cat_NALS_e(i).mx)',w,'g')
 bar3((3*(mx_channels_e_new==5).*cat_NALS_e(i).mx)',w,'c')

 bar3(2*(mx_channels_e_new==1)',w,'k')
 bar3(2*(mx_channels_e_new==2)',w,'b')
 bar3((mx_channels_e_new==0)',w,'w')
```

```
view(2);

xlim([0.5 size(gspes_e,1)+0.5]);
ylim([0.5 size(gspes_e,2)+0.5]);

% Pos = get(gca,'OuterPosition');
% Pos(2) = 0.05;
% Pos(3) = Pos(3)+ 0.02;
% Pos(4) = Pos(4) - 0.25;
% set(gca,'Position',Pos)

set(gca,'Ytick',1:size(gspes_e,2))
set(gca,'YTickLabel',flipud(ylbl_new),'FontSize',Font_size)

set(gca,'XTick',1:size(gspes_e,1))

if exist('ch_list','var')
%set(gca,'XTickLabel',ch_list,'FontSize',Font_size)
set(gca,'XTickLabel',[])
xlbl = num2cell(ch_list);

%ind = zeros(1,length(fig_bold));

for k = 1:length(fig_bold)
%ind(k) = find(fig_bold(k) == ch_list);
xlbl{fig_bold(k)} = ['\color{blue}\bf ' int2str(ch_list(fig_bold(k)))];

end

ind2 = (find(sum( ((mx_channels_e_new==4).*cat_NALS_e(i).mx)',1) > 0));
for k = 1:length(ind2)

if any(ind2(k) == fig_bold)
xlbl{ind2(k)} = ['\color{green}\bf ' int2str(ch_list(ind2(k)))];
```

```
else
xlbl{ind2(k)} = ['\color{red}\bf ' int2str(ch_list(ind2(k)))];
end

end



text(1:size(gspes_e,1),(size(gspes_e,2)+0.75)*ones(1,size(gspes_e,1)),xlbl','HorizontalAli
gnment','center','Interpreter','tex','FontSize',Font_size);

else
set(gca,'XTickLabel',1:size(gspes_e,1),'FontSize',Font_size)
end



%rotateXLabels( gca, 90)
ylabel('Early')
xlabel('Channel')
xlabel_handle = get(gca,'XLabel');
p = get(xlabel_handle,'Position');
p(2) = round(p(2));
set(xlabel_handle,'Position',p)
title([fig_title ' onset']);



save_name = [char(titles{i}) '_early'];
saveas(gcf,[save_name '.fig'],'fig');
saveas(gcf,[save_name '.jpeg'],'jpeg');
close

figure('Name',[char(titles{i}) '_high']);
```

```
screen_size = get(0, 'ScreenSize');
set(gcf, 'Position', [screen_size(3)/4 screen_size(4)/4 screen_size(3)*(2/3)
screen_size(4)*(3/4) ] );



bar3(3*((mx_channels_h_new==6).*cat_NALS_h(i).mx)',w,'y')
hold on
bar3(3*((mx_channels_h_new==4).*cat_NALS_h(i).mx)',w,'r')
bar3(3*((mx_channels_h_new==3).*cat_NALS_h(i).mx)',w,'g')
bar3(3*((mx_channels_h_new==5).*cat_NALS_h(i).mx)',w,'c')

bar3(2*(mx_channels_h_new==1)',w,'k')
bar3(2*(mx_channels_h_new==2)',w,'b')
bar3((mx_channels_h_new==0)',w,'w')

view(2);

xlim([0.5 size(gspes_e,1)+0.5]);
ylim([0.5 size(gspes_e,2)+0.5]);

% Pos = get(gca,'OuterPosition');
% Pos(2) = 0.05;
% Pos(3) = Pos(3)+ 0.02;
% Pos(4) = Pos(4) - 0.25;
% set(gca,'Position',Pos)

set(gca,'Ytick',1:size(gspes_e,2))
set(gca,'YTickLabel',flipud(ylbl_new),'FontSize',Font_size)

set(gca,'XTick',1:size(gspes_e,1))

if exist('ch_list','var')
set(gca,'XTickLabel',[])
xlbl = num2cell(ch_list);
```

```
%ind = zeros(1,length(fig_bold));


for k = 1:length(fig_bold)
%ind(k) = find(fig_bold(k) == ch_list);
xlbl{fig_bold(k)} = ['\color{blue}\bf ' int2str(ch_list(fig_bold(k)))];

end

ind2 = (find(sum( ((mx_channels_h_new==4).*cat_NALS_h(i).mx)',1) > 0));
for k = 1:length(ind2)

if any(ind2(k) == fig_bold)
xlbl{ind2(k)} = ['\color{green}\bf ' int2str(ch_list(ind2(k)))];
else
xlbl{ind2(k)} = ['\color{red}\bf ' int2str(ch_list(ind2(k)))];
end

end


text(1:size(gspes_e,1),(size(gspes_e,2)+0.75)*ones(1,size(gspes_e,1)),xlbl','HorizontalAli
gnment','center','Interpreter','tex','FontSize',Font_size);


else
set(gca,'XTickLabel',1:size(gspes_e,1),'FontSize',Font_size)
end
%rotateXLabels( gca, 90)

ylabel('High')
xlabel('Channel')
xlabel_handle = get(gca,'XLabel');
```

```
p = get(xlabel_handle,'Position');
p(2) = round(p(2));
set(xlabel_handle,'Position',p)
title([fig_title ' onset']);



save_name = [char(titles{i}) '_high'];
saveas(gcf,[save_name '.fig'],'fig');
saveas(gcf,[save_name '.jpeg'],'jpeg');
close


end



% e_spessample (h_spessample)>= NALS (=2std ; so all responses are included)
% e_spessample>= 3std
% e_spessample>= 4std (2NALS)
% e_spessample>= 5std
% e_spessample>= 6std (3NALS)



function
[e_ratio_pc,h_ratio_pc,Sup_h,Dep_h,Sim_h,Fac_h,Sup_e,Dep_e,Sim_e,Fac_e,Error_e,Er
ror_h,e_noresponse,h_noresponse,h_spessample,e_spessample,h_ppessample,e_ppessam
ple] =
classify_data(m_amps_spes,m_amps_ppes,m_indices_spes,m_indices_ppes,NALS,NAL
P,channel_use)



%COMPARISONS - INITIALISING
```

```
e_spessample = zeros(size(m_amps_spes,1),1);
h_spessample = zeros(size(m_amps_spes,1),1);

e_ppessample = zeros(size(m_amps_spes,1),1);
h_ppessample = zeros(size(m_amps_spes,1),1);

e_ratio_pc = zeros(size(m_amps_spes,1),1);
h_ratio_pc = zeros(size(m_amps_spes,1),1);

Sup_h = zeros(size(m_amps_spes,1),1);
Dep_h = zeros(size(m_amps_spes,1),1);
Sim_h = zeros(size(m_amps_spes,1),1);
Fac_h = zeros(size(m_amps_spes,1),1);

Sup_e = zeros(size(m_amps_spes,1),1);
Dep_e = zeros(size(m_amps_spes,1),1);
Sim_e = zeros(size(m_amps_spes,1),1);
Fac_e = zeros(size(m_amps_spes,1),1);

Error_e = zeros(size(m_amps_spes,1),1);
Error_h = zeros(size(m_amps_spes,1),1);

e_noresponse = zeros(size(m_amps_spes,1),1);
h_noresponse = zeros(size(m_amps_spes,1),1);


for j = 1:size(m_amps_spes,1) %defining H AND E SPES AND PPES FROM MAX1,
MAX2, PMAX1 ETC...

 if channel_use(j) == 1

 mx = m_indices_spes(j,1:2);
 ind = find(mx ~= 0);
 [m,ind2] = min(m_indices_spes(j,ind));
```

```
ind2 = find(mx == m);
e_max = m_amps_spes(j,ind2);
h_max = max(m_amps_spes(j,ind));

mn = m_indices_spes(j,3:4);
ind = find(mn ~= 0);
[m,ind2] = min(m_indices_spes(j,2+ind));
e_min = m_amps_spes(j,ind2+2);
h_min = min(m_amps_spes(j,ind+2));

e_spessample(j,:) = e_max - e_min;
h_spessample(j,:) = h_max - h_min;



mx = m_indices_ppes(j,1:2);
ind_mx = find(mx ~= 0);

mn = m_indices_ppes(j,3:4);
ind_mn = find(mn ~= 0);


if ~isempty(ind_mx) && isempty(ind_mn)

[m,ind2] = min(m_indices_ppes(j,ind_mx));
ind2 = find(mx == m);
e_max = m_amps_ppes(j,ind2);
h_max = max(m_amps_ppes(j,ind_mx));
e_min = ((e_max<0)*-2 + 1)*NALP(j,:);
h_min = ((h_max<0)*-2 + 1)*NALP(j,:);

elseif isempty(ind_mx) && ~isempty(ind_mn)
[m,ind2] = min(m_indices_ppes(j,2+ind_mn));
e_min = m_amps_ppes(j,ind2+2);
```

```
h_min = min(m_amps_ppes(j,ind_mn+2));
e_max = ((e_min<0)*-2 + 1)*NALP(j,:);
h_max = ((h_min<0)*-2 + 1)*NALP(j,:);
elseif isempty(ind_mx) & isempty(ind_mn)
e_max = 0;
e_min = 0;
h_min = 0;
h_max = 0;
else
[m,ind2] = min(m_indices_ppes(j,ind_mx));
ind2 = find(mx == m);
e_max = m_amps_ppes(j,ind2);
h_max = max(m_amps_ppes(j,ind_mx));
[m,ind2] = min(m_indices_ppes(j,2+ind_mn));
e_min = m_amps_ppes(j,ind2+2);
h_min = min(m_amps_ppes(j,ind_mn+2));
end

e_ppessample(j,:) = e_max(1) - e_min(1);
h_ppessample(j,:) = h_max(1) - h_min(1);



e_ratio_pc(j,:) = 100*e_ppessample(j,:)/e_spessample(j,:); %CALCULATE RATIOS
h_ratio_pc(j,:) = 100*h_ppessample(j,:)/h_spessample(j,:);


%H DEFINE WHETHER SUPPRESSION, DEPRESSION SIMILAR OR
%FACILITATION


if (h_spessample(j,:) < 0) || (h_ppessample(j,:) < 0)
Error_h(j,:) = 1;
else
```

```
if h_spessample(j,:) < NALS(j,:)
h_noresponse(j,:) = 1;
else
if h_ppessample(j,:) < NALS(j,:)
Sup_h(j,:) = 1;
else
if abs(h_spessample(j,:) - h_ppessample(j,:)) < NALS(j,:)
Sim_h(j,:) = 1;
else
if h_ppessample(j,:) > h_spessample(j,:)
Fac_h(j,:) = 1;
elseif h_spessample(j,:) > h_ppessample(j,:)
Dep_h(j,:) = 1;
end
end
end
end
end
```

```
%E DEFINE WHETHER SUPPRESSION, DEPRESSION SIMILAR OR
%FACILITATION
if (e_spessample(j,:) < 0) || (e_ppessample(j,:) < 0)
Error_e(j,:) = 1;
else
if e_spessample(j,:) < NALS(j,:)
e_noresponse(j,:) = 1;
else
if e_ppessample(j,:) < NALS(j,:)
Sup_e(j,:) = 1;
else
if abs(e_spessample(j,:) - e_ppessample(j,:)) < NALS(j,:)
```

```
Sim_e(j,:) = 1;
else
if e_ppessample(j,:) > e_spessample(j,:)
Fac_e(j,:) = 1;
elseif e_spessample(j,:) > e_ppessample(j,:)
Dep_e(j,:) = 1;
end
end
end
end
end

end
end
```

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